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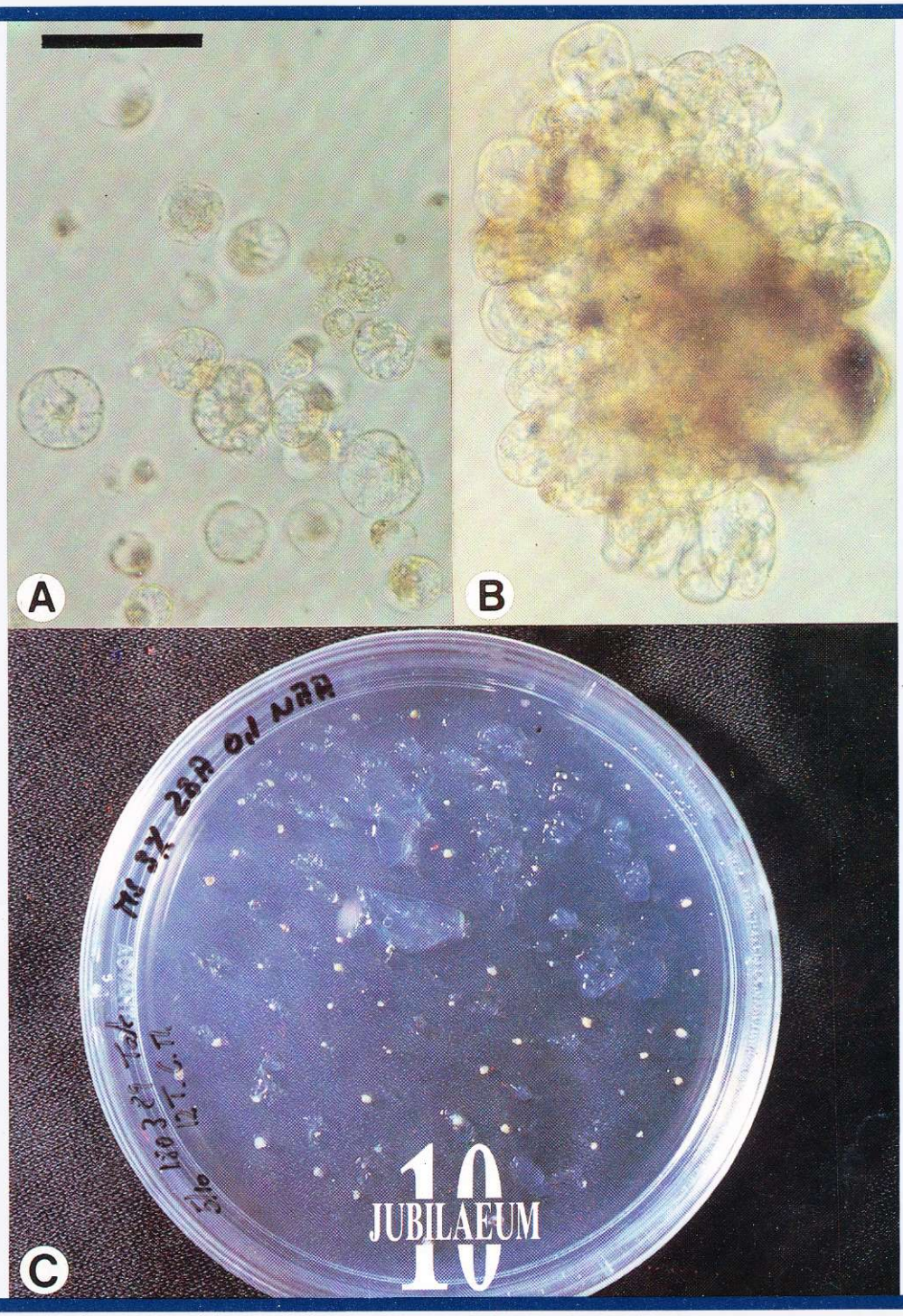

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FAGOPYRUM (Buckwheat Newsletter) is open to everyone interested in buckwheat and will cover all aspects of buckwheat research: genetics, cytology, breeding, cultivation, nutrition, utilization, biochemistry and other. No special priority is given to any language. Scientific papers, reviews, research notes on work in progress or on results not yet published: comments and speculations related to buckwheat; list of stock materials wanted or available; lists of names, addresses and field of work of scientists who have expressed the desire to receive the Newsletter; lists of publications which are related to buckwheat and which have appeared during preceding years; announcements concerning the promotion of research on buckwheat (workshop, symposia, and so on); abstracts and/or contents of published papers/books on buckwheat; bibliographies and other information related to buckwheat or buckwheat research will be published. In order to facilitate the elaboration of the bibliography scientists are asked to send reprints of their own publications to the editor of Fagopyrum.

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Front page photo: Callus formation from protoplasts of *F. tataricum* (see paper of Lachmann and Adachi pp. 62-64).

KAZALO - CONTENTS

Fagopyrum 10 (1990): 5-6

The phenomenon of dominance, competition, compensation and overcompensation in buckwheat productivity

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Key words: ideotype, habitus, productivity

Abstract

Recognition of changes in buckwheat productivity connected with dominance, competition, compensation and overcompensation, as a consequence of discarded branches, inflorescences and leaves, was studied. The most productive was found to be an ideotype of buckwheat with main stem only, without first or second order branches.

Pojav dominance, tekmovalnosti, nadomeščanja in presežnega nadomeščanja pri rodovitnosti ajde

Proučevane so bile spremembe v rodovitnosti ajde, povezane s prevlado, tekmovalnostjo, nadomeščanjem in presežnim nadomeščanjem, ki so bile posledica odstranitve vej, socvetij in listov. Ugotovljeno je, da je najbolj rodoviten ideotip rastlin ajde, ki ima samo glavno steblo brez stranskih vej prvega ali drugega reda.

Synopsis

W pracy przedstawiono zmiany w produktywności gryki związane z dominacją, konkurecją, kompensacją i nadkompensacją jako efekt usuwania gałązek, kwiatostanów i liści. Stwierdzono, że produktywniejsze są ideotypy gryki z głównym pędem, bez gałązek I i II rzędu.

Introduction

In buckwheat productivity, there is a very important relationship between initiation and the amount of blossoming and setting of seeds as yielding organs capacity i.e. their accumulative growth level. The intensity of the generative initiation process and later flower germ growth indicates a typical inductive impulse (Kreft 1986, Listowski 1979, 1985). An intensification of the initiation process and blossoming can be observed together with an increase of the induction cycles or the time factor. This phenomenon was observed in plants with many active growth apex. In various distributions of plant per m^2, a differentiation in light intensity occurs with a corresponding blossoming "eruption". It probably results from a multiplying of growth hormones. Simultaneously, there occurs the phenomenon of competition within inflorescences, between inflorescences and between main stems and branches of different orders (Aufhammer 1980, 1987, 1987a, Listowski 1985, Ruszkowska 1965, Ruszkowski 1965). Moreover, in this case, the high branching which can be observed results in most of the nutrients being used for vegetative growth, and only a small amount is utilized for the accumulation of photosynthetic products (Kreft 1986, Listowski 1979) needed for seed establishment. It is assumed that the course of differentiation in the formation of branches and inflorescences is connected with a concentration of hormone type substances (Kreft 1986, Listowski 1985). The growth of plant mass and assimilation leaf surface is connected with plant structure, as well as the function of structural elements. It was

observed in buckwheat that the sink exerts an influence as a stimulator of the net assimilation rate (Listowski 1979) through intensive growing organs, especially the leaf area, in spite of the ageing of plants.

The main aim of studies has been to identify changes in buckwheat productivity connected with competition, dominance, compensation and overcompensation, resulting from the removal during the vegetation period of the first order branches, inflorescences on the main stem and leaves and first order branches on the main stem.

Material and methods

The study was carried out at Puławy on microplots ($1m^2$). Cultivar Emka. Mineral fertilization: N 60, P_2O_5 54, K_2O 72. Term of sowing 10^{th} May, width of rows 33 cm, distance between seeds in rows 2 cm. During the vegetation period, we removed forming branches, inflorescences and leaves on 20 plants according to the experimental scheme. After harvest we defined the habitus and yield structure of individual plant.

Results

Buckwheat yield structure analyses have shown that the main stem is characterized by the highest productivity, first order branches, and especially ontogenically older branches, lower.

The phenomenon of dominance and competition causes changes in habitus and productivity of buckwheat plants. Discarding first order branches results in lengthening of the main stem and an increase in grain yield. Discarding the main stem results in a significant increase in first order branch productivity.

The phenomenon of compensation and overcompensation occured only in plants in which we removed the inflorescences from the main stem. When a small number of inflorescences was removed, a compensation phenomenon was observed, when a high number - overcompensation.

Changes in buckwheat plant assimilate leaf surface influences the habitus and productivity of the main stem and the first order branches. A typical dominance and competition phenomenon occurs. A decrease in first order branch productivity as a result of changes in leaf assimilate surface causes an increase in the grain yield of the main stem.

The ideotype productivity of a single buckwheat plant is mainly connected with the yield level of the main stem. Deficiency in the main stem cannot be compensated by higher productivity of first order branches.

In genetic and breeding studies, as well as in the construction of buckwheat field architecture, it is very important to get the monotype (only main stem) ideotype of a buckwheat plant. It is essential for increasing the yield capacity of buckwheat.

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Fagopyrum 10 (1990): 7-11

How to combine the reproductive system with biotechnology in order to overcome the breeding barrier in buckwheat

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Key words: *Fagopyrum cymosum*, *F. esculentum*, *F. tataricum*, embryo abortion, embryo culture, embryo-sac, fertilization, heterostylism, incompatibility, interferomicroscopy

Buckwheat proteins have one of the highest known biological values, especially because of the high lysine content. In spite of its high nutritional value, however, for a long time there has been a decline in buckwheat production in all countries (Nešković et al. 1986). In order to meet the increasing demands of rapidly expanding populations in the coming century, efforts must be made to improve the productivity of buckwheat. Major causes of low buckwheat productivity occurring in its reproductive system might include: 1) self-incompatibility caused by dimorphic heterostylism, 2) incomplete reproductive organ, mainly in the female, 3) failure of fertilization, and 4) seed collapse in the post-zygotic stage (Adachi 1986).

I would like to describe here the present status of these experimental problems according to our own research.

Sporophytic incompatibility caused by heterostylism.

F. esculentum and *F. cymosum* have a dimorphic sporophytic system of self-incompatibility. The incompatibility reaction has been recognized to be governed by a complex of genes or a super gene, S (Sharma and Boyes 1961). However, deformed characters can sometimes be observed which must be controlled by subgenes. We induced the autotetraploid to diversify the genotypes by colchicine treatment. The progeny obtained frequently included autogamous homomorphic lines (Adachi et al. 1982). In the case of an illegitimate combination in the diploid, the incompatibility caused by heterostylism was so strong that almost all of the pollen tubes failed to reach the base of the style. In contrast, the inhibition of pollen tubes tends to be weaker in induced autotetraploids, especially in deformed strains PH (Pin-type homostyle) and TH (Thrum-type homostyle). We tried to analyse the genetic composition of heterostylism by using variations in the style. We have reported some results elsewhere. However, a chromosomal irregularity occurs in the autotetraploid during meiosis. Further analysis might be limited in autotetraploids. Recently, in vitro culture and subsequently plant regeneration from callus have been achieved by several researchers. Prof. Kreft has indicated the possible applications of in vitro techniques to buckwheat (Kreft 1983). One of them would be to analyse haploid formation. If we can obtain haploid plants easily by anther culture, the morphological and genetic analysis might be made very easily. Our laboratory and Bohanec (1989) succeeded in anther culture in buckwheat. However, to date these plants have all been diploid. We have reported the anther culture method (Adachi *et al.* 1988). However, real haploids have not yet been successfully obtained.

Another possible challenge might be the use of tartary buckwheat, *F. tataricum* for overcoming heterostylism, which is only one of the self-compatible species in the genus *Fagopyrum*. Cross-incompatibility with common buckwheat is, however, strong with the conventional method. If we could succeed

in fusing the somatic cells of common and tartary buckwheat, this could provide a new prospect not only for the analysis of heterostylism incompatibility but also for an improvement in productivity.

Defectiveness of embryo-sac and failure of fertilization.

Low and unstable seed set of buckwheat depends on environmental conditions, especially high temperature. The main cause of the defect is abnormal development of the reproductive organs. Nakamaura (1949) defined it as an enervative sterility of the pre-zygote under cultivation at high temperature. It is impotence caused by the incomplete development of the embryo sac which might in turn be caused by an imbalance between assimilation and respiration in the floral organs. Adachi and Kajita (1989) reported varietal differences of flower number and seed set along each cluster in autumn and spring seasons as shown in Fig. 1. Lachmann and Adachi (1989) studied varietal responses in the reproductive characteristics, especially flower number and seed set, depending on photoperiodism and thermoperiodism under controlled conditions. On this basis, plant breeders should attempt to decrease the total number of flowers per plant. Less energy would then be wasted in flower formation in buckwheat.

Post-zygotic embryo abortion and embryo culture.

Nakayama (1975) pointed out the genetic control of post-zygotic seed abortion. We suggested that there might be a critical point for embryo growth between 2 and 3 days after pollination. Embryo development after pollination was subsequently followed histochemically by new embedding techniques which were developed in my laboratory. Fig. 2 shows the symptoms indicating embryo abortion. We have recently succeeded in observing embryo development very easily and in detail by Nomalsky's differential interferomicroscopy, as shown in Fig. 3. It could be useful for analysis of seed collapse in the developmental process. By checking proembryonic abortion, Uchinomiya (1990) succeeded in ovule culture just three days after pollination.

It is necessary to continue to devise a breeding strategy for overcoming breeding barriers by using the flood of developments in plant biotechnology.

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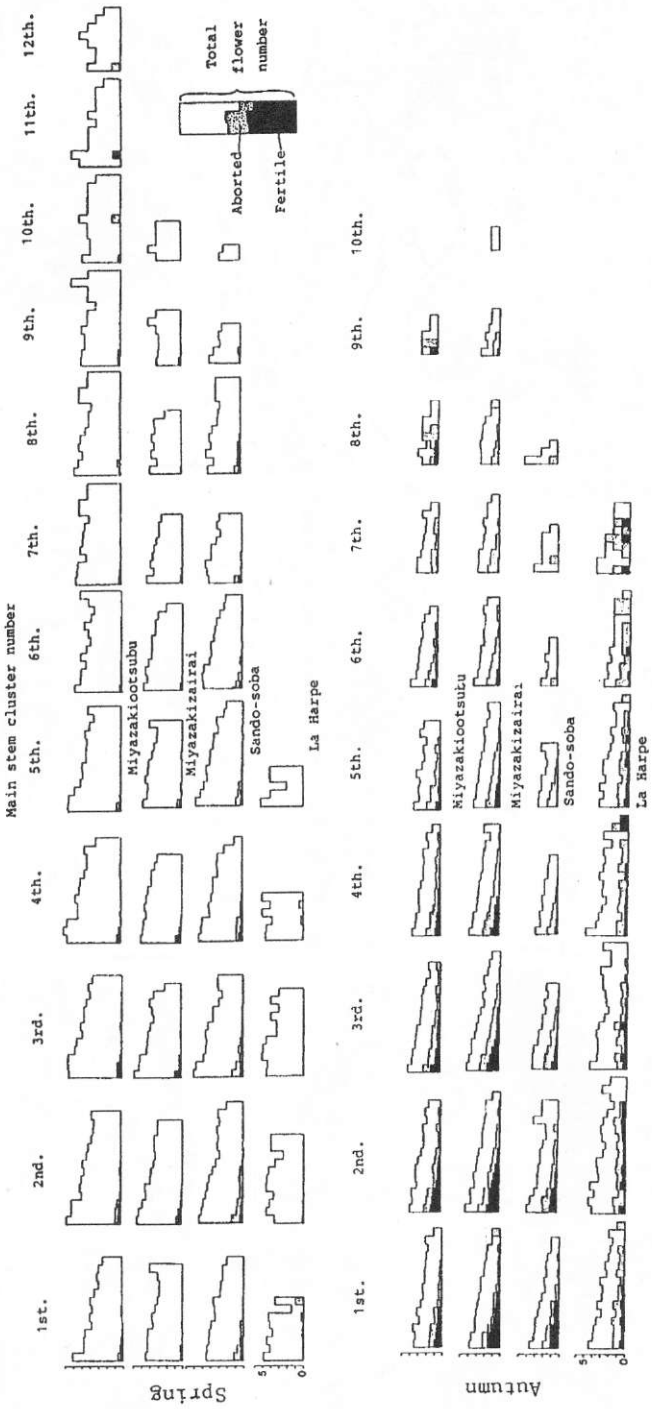

Fig. 1. Flower number and seed set in buckwheat varieties.

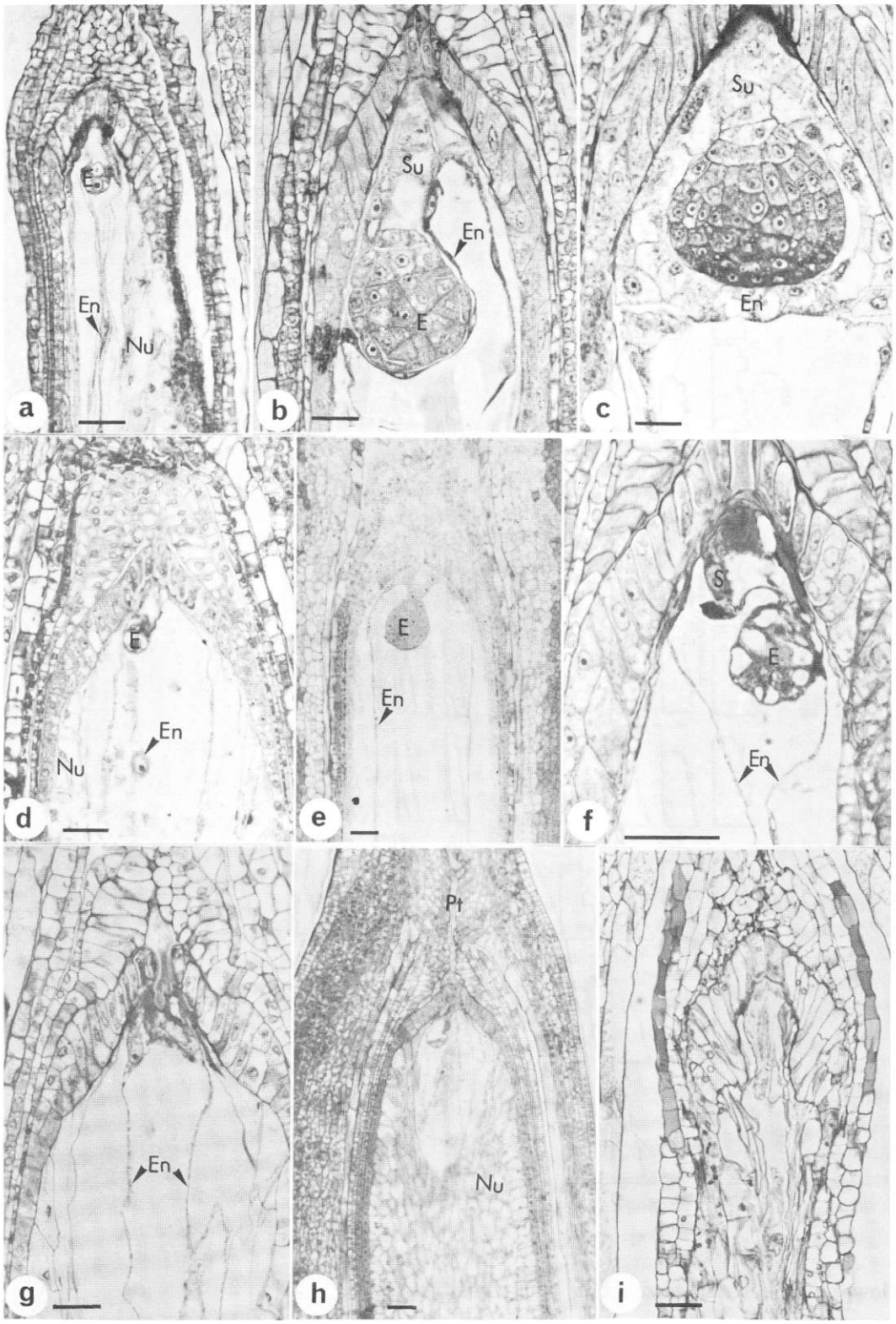

Fig. 2. Embryo development of autotetraploid buckwheat (From Adachi 1986).
a) one day after pollination (normal), b) three days after pollination (normal), c) four days after pollination (normal), d) to i) are abnormal development of embryo or collapse of embryo-sac. Bar represents 50 μm.
E - egg cell, En - endosperm, Nu - nucellus cells, Pt - pollen tube, Su - suspensor

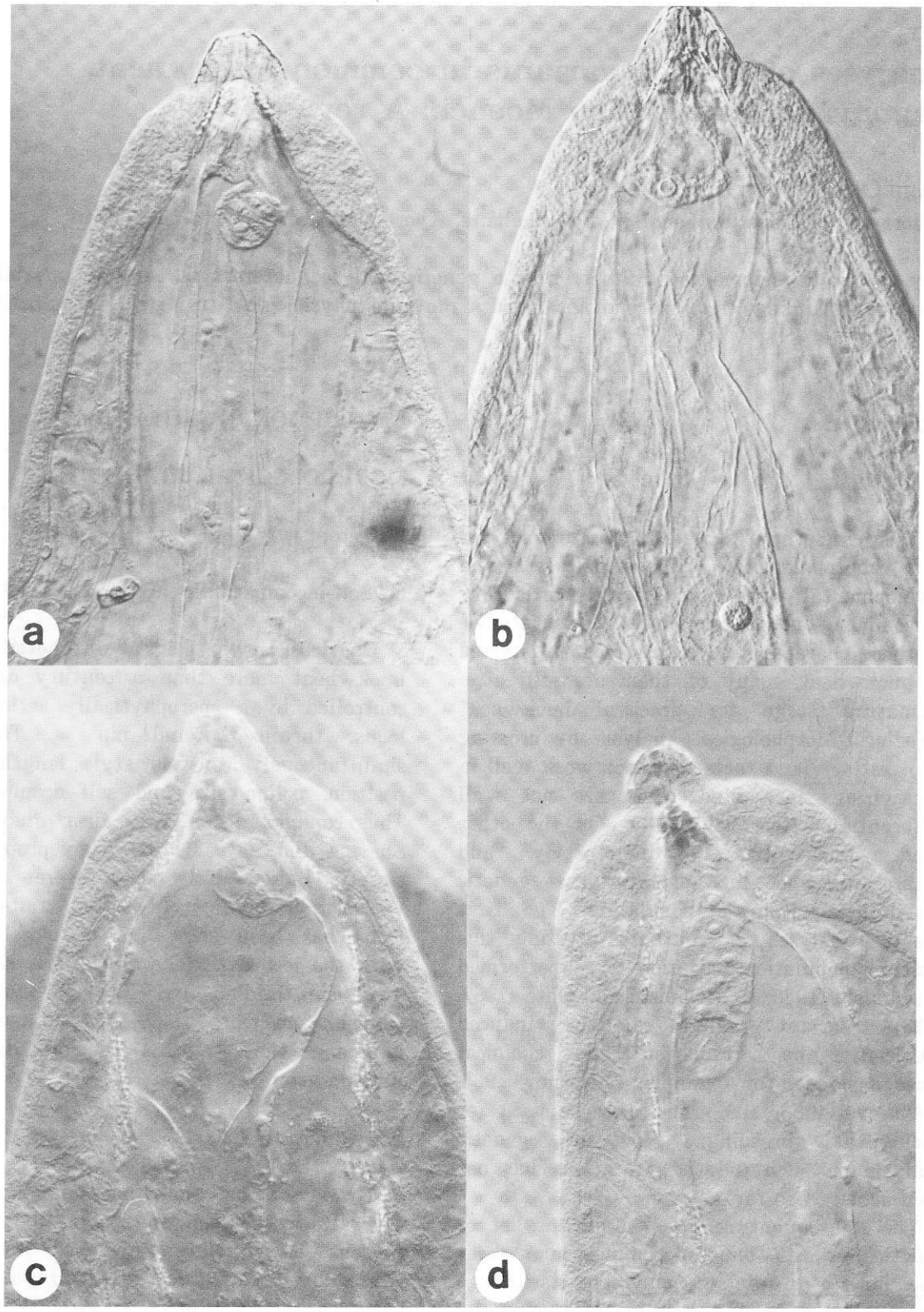

Fig. 3. See-through figure of embryo development by Nomalsky's differential interferomicroscopy. a) to d) are abnormal developments at three days after pollination

Fagopyrum 10 (1990): 12-22

Analyses of genetic variants in common buckwheat, *Fagopyrum esculentum* Moench: A review

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Key words: developmental traits, dwarfism, genetic analyses, heterostyly, isozyme variability, leaf morphology, linkage map, morphological traits, mutants, physiological traits, self-incompatibility

Introduction

This article briefly reviews genetic analyses of morphological, physiological and developmental traits and isozyme variability in common buckwheat. Since genetic studies on these characters have only recently started in buckwheat, many of them are still at a premature stage for practical buckwheat breeding. Morphological analysis by crossing two distinct land races does not work well in buckwheat since land races are not well differentiated morphologically. On the other hand, buckwheat is heterostyly and outbreeding and thus displays great genetic variability within a population. The first genetic analyses of mutants utilizing this trait of population variation were performed by Ohnishi (1986), and Ohnishi and Ohta (1987, 1989). Variant plants can be induced artificially by irradiation and chemical mutagens (see, for example, Zheleznov 1965, Alekseeva 1986, 1989) and may be used for buckwheat breeding. However, genetic analysis on such induced mutations has not been achieved so far.

The subsequent section contains a list of genetic variants found in common buckwheat [the name of mutant (gene symbol, its linkage group)] and a brief description of the results of genetic studies on them. Among the morphological variations listed, vestigial petals (Vp) is the only dominant mutant; all other mutants are recessive.

DESCRIPTION OF MUTANTS

MORPHOLOGICAL TRAITS

1. Heterostyly

(1) Self-incompatibility (S, I)

Charles Darwin pointed out heterostyly in buckwheat more than a century ago. It is controlled by a sporophytically acting single locus: thrum Ss and pin ss. The locus simultaneously controls style length, anther position, pollen size and self-incompatibility. This complex locus was first analyzed by Sharma and Boyes (1961). They proposed five closely linked loci to interpret observed variation in heterostyly-related traits. This S locus has been shown to be closely linked with the loci, dwE (one of the loci controlling dwarfism), Mdh-3 and Est-1 (Ohnishi 1986, Ohnishi and Ohta 1987). The linkage group including the S locus is now designated linkage group I.

2. Dwarfism

Dwarfism in buckwheat is caused by several loci (Ohnishi and Nagakubo 1982); at present six loci are known. Dwarf plants caused by different loci are distinguishable in some cases (e.g. dwA vs. dwD) but not in others (e.g. dwA vs. dwC). The plant height of dwarf plants is highly dependent on the genetic background as well as on the loci involved.

(2) dwarf A (dwA, II)

Dwarf plants are 10-50 cm in height, but they are sufficiently vigorous and fertile. This is one of the most commonly found types of dwarfism in buckwheat and many dwA mutant lines have been isolated from cultivated populations in Japan and other countries (Ohnishi and Nagakubo 1982, Ohnishi 1988a). The dwA locus is closely linked to the loci gsA and ct, and moderately to the Adh locus (Ohnishi 1986, Ohnishi unpublished). This is a good genetic marker for linkage group II.

(3) dwarf B (dwB, II)

This type of dwarfism was first found in a sib-line from the Higashi-Iyayama population in Japan. It is characterized by truncation of the tips of the foliage leaves. The dwarf plants usually grow to more than 50 cm but are less fertile than normal plants. The dwB locus also belongs to linkage group II (Ohnishi 1986).

(4) dwarf C (dwC, III)

This is the dwarfism most commonly found in farmers' fields (Ohnishi and Nagakubo 1982). Plant height, usually 10-70 cm, is highly dependent on the genetic background. It has good viability and similar fertility to normal plants and is therefore a good morphological marker for linkage group III. The dwC locus is closely linked to the py8 and pg6 loci and moderately to the ir, Sdh-1, and Got-2 loci.

(5) dwarf D (dwD, IV)

This form of dwarfism is also frequently found in cultivated populations (Ohnishi and Nagakubo 1982). At the early stage of growth, it has a stem with short internodes and a height of only 10-15 cm, though it later extends less branched stems and reaches 20-60 cm depending on the genetic background. The locus is closely linked to the wl locus.

(6) dwarf E (dwE, I)

This locus is closely linked to the self-incompatibility locus S (Ohnishi 1986). A variety with this dwarf mutant allele has been registered in Canada. Allelism between the allele found in Japan and that found in Canada was confirmed in a cross between them. The dwarf plants have more branches than normal plants, and grow to approximately 40-60 cm in height. They are vigorous, but less fertile.

(7) dwarf F (dwF, II)

This type of dwarf has seldom been found in cultivated populations. The dwarf plants grow to 30 cm at most. The first internode of the plants is almost normal, but the second and latter internodes are extremely short; foliage leaves thus grow compactly near the top of the main stem. The mutant stock isolated from the Kamiagata population in Japan is poorly fertile, although it has normal pollen fertility. The locus is loosely linked to the Sdh-1 and Got-2 loci (Ohnishi and Ohta 1987).

3. Leaf morphology

The common buckwheat leaf is sagitate or triangular. Several mutants affecting leaf morphology have been found, of which cut leaf (ct), irregular cuts (ir), long leaf (lg), miniature leaf (min) and willow leaf (wl) alter the leaf shape, while the mutants crepe leaf (cp) and thick leaf (thl) have a quite different appearance on the leaf surface. Leaf morphology mutants are, in general, good genetic markers unless they suffer from male or female sterility.

(8) crepe leaf (cp, II)

The surface of the foliage leaves is shrivelled like crepe. This mutant was first found in a sib-line of the Kamiagata population in Japan. Another line from the Kim-hae population in Korea was allelic to the cp line mentioned above. This mutant is female sterile in most circumstances, but occasionally has a low fertility, probably depending on the genetic background. This mutant belongs to the second linkage group (Ohnishi and Ohta 1987), but the exact location has not yet been determined.

(9) curled leaf (cu, III)

The mutant has upward curled leaves. It is known to be linked to the Sdh-1 and Got-2 loci, hence belongs to linkage group III (Ohnishi and Ohta 1987). This mutant shows poor fertility, so genetic analyses have not progressed very far.

(10) cut leaf (ct, II)

This mutant was first found in a sib-line derived from the Kim-hae population in Korea. The tips of the foliage leaves have irregular cuts. The mutant plants are completely female sterile, although they have normal pollen fertility. ct is closely linked to the gsA and dwA loci (Ohnishi 1986, Ohnishi and Ohta 1987).

(11) irregular cuts (ir, III)

It was first found in a sib-line originated from the Chilin population in China. A new mutant with a similar phenotype was also found in a homozygous line of gsB (green stem B). The ir locus belongs to linkage group III and lies between dwF and Got-2 (Ohnishi and Ohta 1989). In spite of its distinct abnormality of leaf shape, mutant plants show good fertility in both male and female organs and are easily maintained as a homozygous line.

(12) long leaf (lg, ?)

The phenotype of this mutant is characterized by slightly longer foliage leaves; the width remains almost the same as normal. It is occasionally indistinguishable from normal plants. The linkage relationship has not yet been determined.

(13) miniature leaf (min, VIII?)

The foliage leaves of the mutant line have small leaves, slightly narrower and about 1/2-2/3 the length of normal leaves. When this mutant was first found in a sib-line of an Hungarian population, it had very small short leaves and was thus called 'miniature leaf'. The min locus probably belongs to linkage group VIII since its inheritance appears to be independent of any of the known mutants. However, it remains possible that it is located on the tip of another chromosome (Ohnishi and Ohta 1989).

(14) stuck cotyledons (sc, III)

When the seeds germinate, two cotyledons are stuck together face to face. The mutant plants can later grow foliage leaves, though very weakly. This mutant is very weak and less fertile, but still fertile enough to be kept as a mutant line. It has recently been revealed that the sc locus is linked to the Sdh-1 and Got-2 loci, and hence belongs to linkage group III (Ohnishi unpublished).

(15) thick leaf (thl, VII)

This recessive mutant has thick dark green leaves both in the cotyledons and foliage leaves. Female fertility is slightly depressed. It is closely linked to the Pgm-2 and Mdh-1 loci of linkage group VII (Ohnishi unpublished).

(16) willow leaf (wl, V)

The foliage leaves of this mutant are narrow and long, like a willow leaf. The mutant was isolated from the Kamiagata population in Japan. Mutant plants have normal vigor and normal pollen fertility, but they are almost completely female sterile. It is therefore quite difficult to keep the mutant line by sib-crosses. It was revealed that the wl locus is closely linked with the dwD and Pgi-1 loci, indicating its membership of linkage group V (Ohnishi and Ohta 1987).

4. Stem color

Green stem, pink stem, brown stem and yellow stem are the most commonly found stem color abnormalities. More than three loci are involved in the green stem character in buckwheat; each expresses green color in the stems and branches (Ohnishi and Ohta 1987, 1989). The green stem phenotypes caused by distinct loci are indistinguishable from each other. Pink stem is also controlled by more than one locus. At present only one locus has been identified for the brown stem although

more than one locus will probably be involved (Ohnishi unpublished).

(17) green stem A (gsA, II)

The gsA mutant is the most commonly found type among the different green stem variants (see Ohnishi and Ohta 1987). Mutant homozygotes have clear green stems and branches. The mutants have good viability and good fertility; so this is one of the best morphological markers for any purpose. The gsA locus is very tightly linked with the dwA and ct loci.

(18) green stem B (gsB, II)

This mutant also has clear green stems and branches. The mutant was isolated from the Bosna population in Yugoslavia. It is as vigorous as normal but has quite low fertility. The gsB locus is linked with the rc (red cotyledons) and bw (brown stem) loci, and all together they compose linkage group VI (Ohnishi and Ohta 1989).

(19) green stem C (gsC, II)

This is another stem color mutant displaying clear green stems and branches. Two mutually allelic mutant lines have been established from inbred lines, one from the Geihoku population in Japan and one from an Hungarian population. This is not a good marker since the mutant homozygotes are almost completely female sterile. The gsC locus also belongs to the linkage group II, but is located far from dwA, ct and gsA (Ohnishi and Ohta 1987).

(20) green stem D (gsD, VII)

The mutant was isolated from the Syabrubensi population in Nepal. The stem color is dull green or yellowish green, slightly different from other green stem mutants (gsA - gsC) although in many circumstances all green stem mutants are indistinguishable from each other. Its linkage with the Pgm-2, Dia-1 and 6-Pgdh-1 loci has recently been clarified (Ohnishi unpublished).

(21) pink stem (ps, VI)

The mutant plants have clear pink stems and branches. The pink stems are physically weak so the mutant plants often suffer from lodging. The mutant plants have excellent fertility so the ps locus is a good morphological marker for experimental purposes. When the ps mutant is combined with any of the green stem mutants, the double mutant displays colorless stems. The ps locus is closely linked with the vestigial petal Vp mutant (Ohnishi 1986).

(22) pink stem B (psB, III)

The mutant displays dark pink stems at the early stage of development, and later becomes nearly normal in color, but less vigorous. This mutant has quite low female fertility, and is hence difficult to keep as a homozygous line. It is linked with the py8, Sdh-1 and Got-2 loci in linkage group III. However, the exact location of it has not been determined so far (Ohnishi and Ohta 1989, Ohnishi unpublished).

(23) pink stem C (psC, ?)

The pink stem mutant isolated from an Hungarian population by a sib-cross was not allelic to any of ps, psB and psD (Ohnishi and Ohta 1987). However, no further genetic analysis has been achieved.

(24) pink stem D (psD, I)

Although this mutant is called "pink stem", it actually has pale yellowish pink stems, and is quite different in stem color from the other pink stem mutants. The stems of the mutant always elongate at the early stage of development and often suffer from lodging. The mutant plants have fewer branches and fewer flowers, and hence set fewer seeds. This mutant psD locates near the S locus of linkage group I (Ohnishi and Ohta 1989).

(25) brown stem (bw, V)

The mutant has brownish stems; the stem color is usually distinguishable from that of the other stem color variants and normals, but occasionally indistinguishable from normal color. The brown mutant isolated from the Higashi-Iyayama population in Japan is closely linked with the red cotyledon (rc) and green

stem B (gsB) loci (Ohnishi and Ohta 1989). Other locus or loci than the bw mentioned above are probably involved in the same brown stem phenotype.

(26) yellow stem (ys, II)

This mutant is characterized by brownish yellow stems. The yellow stems of mutant plants elongate at an early stage of development, like the psD mutant. No detailed linkage analysis has been yet achieved, but a preliminary analysis indicates that the ys locus is linked to the dwA locus, hence it belongs to linkage group II (Ohnishi unpublished: data on dwA x ys given in the footnote of Table 3 of Ohnishi and Ohta 1987 are incorrect).

5. Leaf color mutants

Mutants in this category, with the exception of red cotyledon mutant (rc), which has extra red pigmentation in the cotyledons, are chlorophyll-deficient. Ohnishi (1982) pointed out that more than 100 loci are probably involved in chlorophyll-deficient phenotypes. Mutants have been classified for the sake of practical convenience into albino, yellow, pale yellow, pale green and variegated. Albino and yellow mutants have an unambiguous clearcut phenotype. Genetic analyses on them are, however, very difficult since they are lethal and maintenance of these mutants in a heterozygous condition is impracticable. The variegation of leaf color has attracted the attention of buckwheat breeders. Some variegation mutants segregate as a Mendelian factor (see Ohnishi 1982), but others exhibit a rather complicated manner of inheritance (Ali-Khan 1971, Tatebe 1972). Variegation mutants do not, therefore, make good genetic markers. Mutants analyzed for their linkage relationship are thus mostly the pale green or pale yellow types, which are not lethal.

(27) red cotyledons (rc, VI)

The cotyledons of this mutant are dark red when they are extended after germination. The leaf color later becomes almost normal though the red coloration remains in the stem, branches and flowers. The vigor and fertility of the mutant are normal, but it has a weaker root system. The rc locus is linked with the bw and gsB loci (Ohnishi and Ohta 1989). The double mutant gsB rc homozygote has creamy green stems and this phenotype is distinguishable from gsB and rc as well as from normal.

(28) pale green No. 6 (pg6, III)

A mutant with pale green foliage leaves was isolated from the Niimi population in Japan by sib-cross. This mutant, pg6, is closely linked with dwC and Got-2 (Ohnishi and Ohta 1987).

(29) pale yellow No. 8 (py8, III)

The mutant has pale yellowish cotyledons and foliage leaves. It is distinguishable from normal plants throughout all stages of development. It has good viability and fertility and so is an exceptionally good genetic marker. It is closely linked with the dwC locus and moderately with the Sdh-1 and Got-2 loci (Ohnishi and Ohta 1987).

(30) pale yellow No. 12 (py12, III)

A pale yellow mutant was isolated from an Hungarian population, and its close linkage to the dwC locus was revealed by Ohnishi and Ohta (1987). That is all we know so far about this mutant.

(31) pale yellow No. 22 (py22, III)

This mutant was isolated from the Che-ju population of Korea. Both cotyledons and foliage leaves have a pale yellow color. Ohnishi and Ohta (1987) reported that it is moderately linked to the dwC, Sdh-1 and Got-2 loci.

(32) pale yellow No. 23 (py23, III)

This mutant was isolated from the Kamiagata population in Japan. The mutant has a pale yellow color in both the cotyledons and foliage leaves. It has been demonstrated that the py23 locus is linked with the Sdh-1

locus (Ohnishi and Ohta 1987) and the Got-2 locus (Ohnishi unpublished). It thus clearly belongs to linkage group III.

(33) pale yellow No. 24 (py24, VII?)

The mutant has clear yellow to pale yellow cotyledons and foliage leaves. The foliage leaves of the mutant are also apparently thinner than normal leaves. The plants have weaker viability and lower fertility. Although it has been suggested that the py24 locus is distantly linked to the Pgm-2 locus (Ohnishi and Ohta 1987), this conclusion is questionable (Ohnishi unpublished).

(34) pale yellow No. 25 (py25, V)

This pale yellow mutant, isolated from the Yarsa population in Nepal, exhibits a pale yellow color in both the cotyledons and leaves. It has now been shown to be linked to the ps locus, indicating its membership of linkage group V (Ohnishi unpublished).

6. Other morphological characters

(35) vestigial petals (Vp, V)

The gene symbol Np was used for this mutant in Ohnishi (1986). This is a dominant mutant; all other morphological mutants so far found in buckwheat are recessive. This mutant was first found as a mutant plant in a farmer's field in Niimi, Japan. The flowers of both the heterozygotes Vp/+ and the homozygotes Vp/Vp have vestigial petals and look like petalless flowers. However, the mutants occasionally have white vestigial petals. The expression of Vp probably depends on the genetic background. Both the homozygotes and the heterozygotes are viable and fertile. It is linked to the ps locus (Ohnishi 1986).

(36) determinate growth habit (det, ?)

This mutant plant has a determinate rather than the normal indeterminate growth habit. It was first found by Fesenko (1968) and later independently by Bohanec and Kreft (1981). The frequency of the det gene has been found to be very high in such cultivated populations as the Ticino population in Italy and several Yugoslav populations (Ohnishi unpublished). The det gene has pleiotropic effects: cessation of further growth of the apex and new leaves and the formation of a unique morphology of the inflorescence (see Kreft 1989). It has been used for improving cultivars with respect to uniform ripening, resistance to lodging and constant yield. The expression of the det gene highly depends on the genetic background, and it is therefore often difficult to distinguish these mutant plants from normal plants. As a result, genetic analysis of this mutant has not progressed.

(37) bushy inflorescence (bs, III?)

When the mutant plants mature, short branches clump from the uppermost part of the main stem, like the dwF plants. The leaf and stem color of the mutants are slightly paler than in normal plants. The mutant plants are thus distinguishable even at an early stage of development. Since the mutant homozygotes are almost completely female sterile, linkage study of the mutant has not progressed very far. However, a preliminary analysis indicates that it belongs to linkage group III (Ohnishi unpublished).

I now briefly comment on other morphological traits. Morris (1947) reported that winged seeds are dominant over non-winged ones, although inheritance of the trait was rather complicated. Apparently two types of winged condition are present in buckwheat; one has thick broad wings and is often found in land races from the northern part of temperate Asia, i.e. Japan and northern China. The other type is found mainly in southern China and has faintly-notched thin wings around the edges of the kernels (Ohnishi unpublished). No detailed study has been made on these variants. Flower color is also variable among different land races as well as among individuals in a population. Most plants in Asia have pale pink flowers, while Japanese and Korean land races have white flowers. Seed color is also known to be variable among

land races; the grey buckwheat races found in southern Europe, Yugoslavia, Italy and France, are remarkably beautiful (Kreft 1981). Unfortunately, genetic studies have not been performed on the color of seeds and flowers.

ISOZYME LOCI

Electrophoretic analysis of isozymes in buckwheat was started in 1983 in my laboratory. The main purpose was a world-wide survey of allozyme variability (see Ohnishi 1988b). Linkage analyses of isozyme loci, particularly of those loci showing polymorphism, can easily be done by analyzing F_2 progeny (Ohnishi 1986). Furthermore, combining isozyme loci and morphological traits, linkage analyses have greatly progressed in a short time (Ohnishi and Ohta 1987, 1989). The method of electrophoresis employed with buckwheat is standard, so see for example Tanksley and Orton (1983) and Ohnishi and Nishimoto (1988).

(38) Alcohol dehydrogenase (Adh, II)

Only one locus is known to be involved in buckwheat Adh, although two loci are usually known in Adh in other plant species. The enzyme is only active in the seeds; after germination, activity quickly decreases in the leaves and other tissues. It has now been shown that the Adh locus is closely linked with the gsA and dwA loci, indicating its membership of linkage group II (Ohnishi unpublished).

(39) Diaphorase (Dia-1, ?; Dia-2, VII)

Diaphorase is controlled by two independent loci, designated by Dia-1 and Dia-2. The Dia-2 locus controlling the slower band is polymorphic in most Chinese and Nepali populations (Ohnishi and Nishimoto 1987, Ohnishi 1988b). The locus is linked with the gsD, Pgm-2, 6-Pgdh-1 and Mdh-1 loci (Ohnishi and Ohta 1989). The Dia-1 locus is completely monomorphic in all populations so far studied, so no genetic analysis has been carried out.

(40) Esterase (Est-1, I; several unidentified loci)

Many loci are involved in esterase in buckwheat, as in other plant species. The electrophoresis methods employed with buckwheat, however, do not clearly separate esterase isozymes. Only the fastest band has therefore been analyzed so far. It is controlled by an Est-1 locus which is linked with the S, psD and Mdh-3 loci (Ohnishi and Ohta 1989).

(41) Fumarase (Fum, II?)

Experimental band patterns show that only one locus is involved in this enzyme in buckwheat. It is completely monomorphic in all known populations in the world and so no detailed genetic analysis has been performed. However, a preliminary experiment using a variant plant shows its probable location in the second linkage group (Ohnishi unpublished).

(42) Glutamate dehydrogenase (Gdh, ?)

Judging from observed band patterns, this enzyme is controlled in buckwheat by one locus. Although it shows allozyme variability in a few populations in Asia (see Ohnishi and Nishimoto 1987, Ohnishi 1988b), no detailed genetic study has been carried out.

(43) Glutamate-oxaloacetate transaminase (= Aspartate aminotransferase) (Got-1, ?; Got-2, III)

At least two and probably three loci are involved in Got in buckwheat. The Got-1 locus which controls the fastest band zone is almost completely monomorphic; so no attempt has been made to reveal its linkage relationship. The Got-2 band is more active in the leaves than in the seeds. This locus is polymorphic and has three alleles in most populations. The Got-2 locus is closely linked with the dwC, pg6, pg8, Lap and Sdh-1 loci (Ohnishi and Ohta 1987, 1989). Along with the Sdh-1 locus, this is a good marker for linkage group III.

(44) Isocitrate dehydrogenase (Idh, VII)

This enzyme is also controlled by one locus.

The enzyme is dimeric, hence three bands appear in heterozygotes, but in homozygotes twin bands appear, one main band and one ghost. Utilizing a mutant isolated from the Dunche population in Nepal, a preliminary analysis was carried out and the result shows that the Idh locus is linked to the thl locus, indicating its membership of linkage group VII. (Ohnishi unpublished).

(45) Leucine aminopeptidase (Lap, III)

Lap shows monomeric band patterns. It is completely monomorphic in all land races so far examined. Using a variant plant, it was shown that the locus is linked to the py8 Sdh-1 loci (Ohnishi and Ohta 1989).

(46) Malate dehydrogenase (Mdh-1, VII; Mdh-3, I; Mdh-4, I?)

Three loci have been analyzed; however, judging from the band patterns which have appeared, at least two more loci are probably involved in Mdh in buckwheat. The Mdh-1 locus which controls the fastest band zone in Mdh is highly polymorphic in all known populations of the world (Ohnishi 1988b). The locus is closely linked with Pgm-1, 6-Pgdh-1 and gsD (Ohnishi and Ohta 1989). The locus name of "Mdh-2" has tentatively been given to the second fastest band zone in Mdh. However, it always shows the same band patterns as Mdh-1, which may imply that the Mdh-2 band is just an experimental artifact. No further analysis has been done on it. The Mdh-3 locus controls the most active third band in Mdh. The Mdh-3 locus is polymorphic in most populations. The locus is closely linked with the S, psD and dwE loci (Ohnishi and Ohta 1987, 1989). The Mdh-4 band overlaps with the Mdh-3 band if the N or S alleles segregate at the Mdh-3 locus. On the other hand, if the Mdh-3 locus has the F allele, the Mdh-4 bands do not overlap with the Mdh-3 band, and the Mdh-4 locus can easily be analyzed. Preliminary study indicates that the Mdh-4 locus is linked with the S locus (Ohnishi unpublished).

(47) Phosphoglucomutase (Pgm-1, ?, Pgm-2, VII)

Pgm is controlled by two independent loci in buckwheat as in many other plant species. The slower band is controlled by the Pgm-2 locus, which is highly polymorphic in all known populations in the world except populations in Kashmir, India (Ohnishi and Nishimoto 1988). The Pgm-2 locus is tightly linked to the 6-Pgdh-1, and also moderately with the Dia-2, Mdh-1 and gsD loci (Ohnishi and Ohta 1987). The Pgm-1 locus is usually monomorphic, so genetic analysis has not been carried out.

(48) Phosphoglucoisomerase (Pgi-1, V; several unidentified loci)

Electrophoresis of Pgi did not yield clearcut bands on the anodal side, so only the band zone on the cathodal side has been analyzed. It is monomorphic. Using a variant plant, the Pgi-1 locus has been shown to be linked with the wl locus (Ohnishi and Ohta 1989) and the dwD locus (Ohnishi unpublished).

(49) 6-Phosphogluconate dehydrogenase (6-Pgdh-1, VII; 6-Pgdh-2, ?)

Two loci are involved in this enzyme in buckwheat. The 6-Pgdh-1 that controls the faster band is highly polymorphic in most populations in China and Japan (Ohnishi 1988b). It is closely linked with the Pgm-2 locus and moderately with the Dia-2 and Mdh-1 loci (Ohnishi and Ohta 1989). The pattern of the second 6-Pgdh-2 band varies at different stages of germination and early development; genetic analysis of the locus is therefore quite difficult.

(50) Sikimate dehydrogenase (Sdh-1, III; Sdh-2, ?)

This enzyme is also controlled by two loci. The Sdh-1 locus which controls the faster band is highly polymorphic in all populations. It is closely linked with the isozyme loci Got-2 and Lap and the morphological markers dwC and dwF. The second locus, which is less active, has not been analyzed because it is entirely monomorphic. This enzyme can be

assayed both in leaves and seeds. Sdh-1 is thus a good genetic marker in linkage studies.

PHYSIOLOGICAL AND DEVELOPMENTAL CHARACTERS

Photoperiod

Buckwheat is a short-day crop, and is therefore grown in summer to autumn. When it is grown in spring or early summer, it grows very tall but has fewer flowers and sets essentially no seed. Such physiological aspects of photoperiod in buckwheat have been extensively investigated since they are of practical importance for cultivation and breeding of buckwheat (see for example Lachmann et al. 1989, Cai et al. 1989, see also Skok and Scully 1955). Nagatomo (1962) studied the dark period necessary for flower initiation and demonstrated that a ten hour dark period is necessary for flower formation in autumn type (short-day) buckwheat and nine hours in summer type (day-neutral) buckwheat. Furthermore, he suggested that the summer type is dominant over the autumn type. This is a rather unexpected result, since the autumn type seems to be "wild"; his results should be confirmed by future studies.

Developmental factors

Earlier and more uniform ripening of kernels is necessary for improving the yield in buckwheat. Earliness or lateness in ripening is a trait controlled by polygenes and possibly also major genes. No statistical analysis has been attempted on this trait. (The same is true of other quantitative characteristics; hence quantitative traits have not been included in this review). A mutant which considerably retards development and flowering was found in a sib-line of the Togakushi population in Japan. A recessive single gene is responsible for this mutant. No further study, however, has been done so far. Similar mutants, giants, have been found by Alekseeva (1989), after irradiation (see also Bochkareva 1989). At one time, a group of buckwheat scientists at Shinshu University tackled a variant which causes seed abortion. However, they did not succeed in isolating and analyzing the seed abortive mutant.

Lethality and sterility mutants

Lethal mutants have been studied in many plant species. They are incidentally found by detection of a skewed segregation ratio of the marker genes. In buckwheat, a lethal factor has been detected closely linked to the dwD locus, but detailed study on it has not been performed (Ohnishi unpublished). Ohnishi and Katayama (1981) surveyed male and female sterility mutants segregating in cultivated populations. They reported that approximately 20 genes are involved in total in male sterility and another 20 genes in female sterility. However, the maintenance of male and female sterility mutants in the laboratory is very tedious, so genetic study on them is impracticable at present.

Disease resistance

Fungal or viral disease has not been studied very much in buckwheat. Research has just started (see example Milevoj 1989, Bobkova and Polivanova 1989). Systematic study of disease resistance in buckwheat must at present focus on the isolation of different fungal and viral strains and of disease resistant and susceptible buckwheat races.

In conclusion, an up-to-date linkage map of *Fagopyrum esculentum* is presented in Figure 1. Many of the loci studied are known only by their linkage group, their exact locations on the chromosomes have not been determined. Furthermore, many mutants, particularly those induced by irradiation and chemical mutagens in the Soviet Union have not been genetically studied. Further studies will be required to construct a detailed linkage map that can be used in buckwheat breeding.

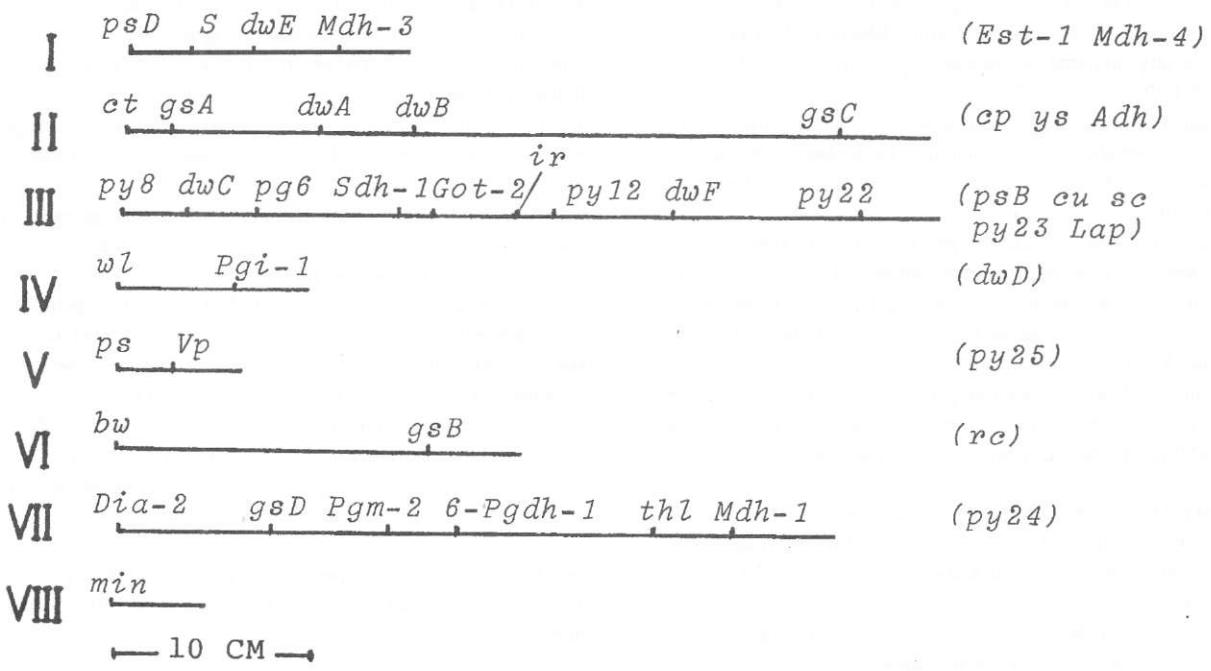

Figure 1. Linkage map of common buckwheat. Approximate positions of the loci are shown; the position of loci shown in parentheses have not yet been determined.

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Pollination biology and reproductive ecology for improving genetics and breeding of common buckwheat, *Fagopyrum esculentum* (1)

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Key words: breeding system, day length, environment, evolution, fitness, heterosis, heterostyly, reproduction, seed production, self-compatibility, self-incompatibility, temperature

Abstract

Common buckwheat (*Fagopyrum esculentum* Moench) is one of the most typical cultivated plants whose flowers display dimorphic heterostyly and self-incompatibility. In general, this plant requires legitimate pollination between different flower types, pin and thrum. The present paper briefly reviews the development of knowledge of the pollination biology and reproductive ecology of buckwheat for the purpose of breeding improvement, seed growth and cultivation technologies on the basis of the nature of the breeding system and its evolutionary significance, and which has resulted in increased heterosis, adaptibility in seed yield and genetic diversity.

Biologija oprašitve in ekologija razmnoževanja za požlahtnitev ajde

Ajda ima med poljščinami najbolj značilen dimorfizem cvetov in samoinkompatibilnost. Rastlina potrebuje za uspešno oprašitev pelod z rastline drugega tipa. V tem članku je kratek pregled biologije oprašitve in ekologije razmnoževanja ajde, da bi tako prispevali k žlahtnjenju, semenarstvu in izboljšanju pridelovanja ajde. K vsemu temu lahko prispevajo raziskave načina razmnoževanja ob upoštevanju evolucije; možno bi bilo povečati heterozo, adaptabilnost, genetsko raznolikost in pridelek zrnja.

Introduction

Knowledge of the so-called pollination and/or reproductive biology of any cultivated species is the most important and practical science for establishing useful plant breeding methods as well as for clarifying the process of species and varietal differentiation in the plant kingdom.

Common buckwheat (*Fagopyrum esculentum* Moench) is one of the most typical cultivated plants whose flowers display dimorphic heterostyly and self-incompatibility (Darwin 1876). Although buckwheat seed is a very valuable and widely used food in the world, systematic breeding research has not so far been carried out except in the USSR, Poland, etc. There are therefore many phenomena in the buckwheat life cycle which remain unknown.

In the USSR, mass selection of buckwheat was started in 1917 at the Shatilov (now Kursk) Station and a leading variety named 'Bogatyrj' (Hero) of high nutritive quality and large grain was released (Kopeljkievskii 1937). The author simultaneously provided a great deal of important information on the pollination biology and reproductive biology of buckwheat which he had discovered through his breeding research work: cross-pollination occured both by wind and insects, special isolation was therefore needed for breeding work; an improved variety could be produced in seven years; by selecting only the largest seeds of a population a yield increase of as much as 44% was obtained; a new type of flower in which the pistil and stamens were of equal length had been found in a number

of individuals alongside the ordinary heterostylous forms; inbreeding by self-pollination had been found to be quite possible and dwarfs and other abnormalities were isolated in inbred generations; a marked reduction in yield was observed in the first inbred generation but yields progressively increased again in later generations.

Omljchenko (1940) reported that buckwheat is very sensitive to environmental conditions and best yields are obtained from varieties when grown in their own locality; the main way of obtaining higher yields from buckwheat is by selection of varieties suited to the region of growth, combined with good cultivation under favourable weather conditions; selection and breeding of new high yielding varieties should be based primarily on local forms or varieties from ecologically similar regions.

Krotov (1944) also performed basic studies on samples of seed from crops sown on large isolated areas and compared them with samples which were the product of one to four years of cross-pollination among different varieties of buckwheat. He concluded that cross-pollination tended to increase the grain yield, but that this was most pronounced in varieties with a low yield and least pronounced (sometimes a decrease) in high yielding varieties; and the increase became larger as successive pollinations became more frequent. The decisive influence, however, was exerted by the conditions which prevailed during the growth of the parent plants and during the period of pollination; the length of the growth period, certain morphological characters, and the quality of grain were also influenced by cross-pollination.

On the other hand, selective fertilization and/or sexual selection has the great advantage of improving various plant species through gametophytic competition (Mulcahy et al. 1983). In terms of selective fertilization in buckwheat, Prezent (1940) reported that the modern Darwinian view postulated a mutual action between pollen and ovule in which the most compatible combination was selected. This was only the best combination from the agricultural point of view, however, when the biological and agricultural aspects coincided; in view of the mutual nature of the selective process, as great a range as possible of both mother plants and pollen parents should be made available in all cases. Best results would be attained where the two parents responded well to the same environmental conditions. So in intravarietal crossing, local varieties are best for use as mother plants whereas when breeding for some new set of conditions, wide crossing would be recommended in order to provide the widest range of possible new adaptations.

The study of pollination biology and evolution should be encouraged as a means of improving practical methods of breeding and seed growth in allogamous crops like buckwheat (Namai and Ohsawa 1986, 1988).

The above descriptions clearly indicate the importance of the nature of the reproductive system of buckwheat in explaining the often inadequate yields and in explaining the different experience of breeders and other workers with varied materials and in different geographical localities. This knowledge is also applicable to current efforts to improve breeding methods.

The present paper is a brief review of the results of buckwheat breeding research by many authors, mainly in the USSR, based on pollination biology and reproductive ecology.

1. General meaning of pollination biology and reproductive ecology

Charles Darwin wrote several books and papers on pollination systems and plant reproduction and many of his conclusions are still valid today. Real (1983) reported that pollination systems have long been recognized as a model for understanding the interplay between natural selection and evolution, and much of the early research in pollination biology, such as long lists of visitors to flowers, individual breeding systems and mechanisms of pollen transfer guaranteeing specific cross-pollination, was only devoted to describing these adaptations and the selective

forces presumed to bring them about. In recent years, however, a remarkable change has taken place in the style of research on pollination biology, which has started to investigate various components of plant-pollinator interactions (Jones and Little 1983), and instead of the peculiarities of the pollination system, emphasis is now placed on uncovering the general principles that underline all ecological and evolutionary changes (Real 1983).

According to three excellent monographs on pollination biology and reproductive ecology (Real 1983, Jones and Little 1983, Doust and Doust 1988), pollination biology consists of the following elements:

a) role of insect pollination in the ecology and evolution,
b) pollinator-plant interactions and the evolution of breeding systems,
c) male competition, female choice, and sexual selection,
d) pollination ecology, plant population structure, and gene flow,
e) foraging behavior of pollinators,
f) adaptive nature of floral traits,
g) evolution, maintenance, and loss of self-incompatibility,
h) sex determination,
i) competition and facilitation for pollination among plants,
j) patterns of fruit and seed production,
k) systems ecology of pollination systems, and
l) applied pollination biology.

Reproductive ecology cosists of: (a) sociobiology of seed, (b) inclusive fitness, seed resources, paternity and maternal allocation, (c) plant morphology and reproduction, (d) influence of competition on plant reproduction, and (e) all the elements of pollination biology.

I believe that a new science called "reproductive biology" or "breeding systematology" should be constructed soon by combining pollination biology and reproductive ecology and that it should become the central focus in evolutionary and agronomic research, as well as in the practical development of agricultural technologies for seed production of various crops.

In the following section, some of the above-mentioned elements especially relevent to the pollination biology and reproductive ecology of buckwheat will be briefly described.

2. Role of insect pollination in the evolution of buckwheat

The main purpose of studying this element is the elucidation of the possible origin of insect pollination and the nature of early insect pollination mechanisms, bearing in mind the importance of insect pollination mechanisms in the establishment of current angiosperm diversity. It is widely known that the relation between flowers and insect pollinators is typically the result of long and intimate coevolutionary interactions. Following the origin of insect pollination, it seems likely that selective pressure for increasing the efficiency of insect pollination would have become significant, and if male and female reproductive structures (pollen grains and ovules) served to attract insects, the percentage of successful pollinations would have been related to the average time between a pollinator's visit to an ovule and its last visit to a pollen-bearing organ of the appropriate species (Crepet 1983). Insect pollination has such obvious advantages at species and at taxonomic levels in the plant kingdom as 1) energy conservation through reliable directional pollination (Cruden 1977), 2) the potential of more outcrossing than wind pollination, especially in populations of relatively widely dispersed individuals (Regal 1977), 3) successful pollination under conditions unfavorable for wind pollen dispersal (Crepet 1983). Pollination by faithful pollinators might provide a mechanism for restricting gene flow from parental populations to isolated demes, thus augmenting the speciation process in a way consistent with the rapid evolution of new species (Grant 1949, Crepet 1983).

Turning attention to the present state of

plant species in *Fagopyrum*, there are three main species, i. e., common buckwheat (*F. esculentum*), tartary buckwheat (*F. tataricum*) and perennial buckwheat (*F. cymosum*). In terms of their pollination requirements, ordinary common buckwheat and perennial buckwheat are generally self-incompatible and cross-pollinated by various insect visitors whereas tartary buckwheat is completely self-compatible and self-pollinated, and infrequently visited by insects. In addition to these phenomena, Marshall (1969) and Astafjev (1974b) described some wind pollination in buckwheat, although it was estimated that the actual intensity of wind pollination is unlikely to be high (Namai 1986, 1989). The green flower of tartary buckwheat, generally without nectar, is unattractive to bees, whereas the white and/or reddish ones of common and perennial buckwheats, producing a lot of nectar, are extremely attractive to them. Even in common buckwheat, a green flower form occasionally occurs under normal crop conditions as well as in intervarietal hybrids and in irradiated materials (Alekseeva 1975, Alekseeva at al. 1988), and in inbred lines (Fesenko 1983). Such plants showed three types of pollination: chasmogamy, partial chasmogamy and partial cleistogamy (Alekseeva 1975).

Heslop-Harrison (1959) revealed that a combination of asexual (apomixis and vegetative) and sexual reproduction gives a plant optimal versatility since sexual reproduction produces new ecotypes and asexual perpetuates the successful ones. Apomixis, which is considered to be the final step in the reduction of pollination, has been described as occuring occasionally in common buckwheat, and stable apomictic populations have already been produced by breeding homozygous lines for genetic factors controlling the ability of the egg cells to develop without fertilization etc. (Zheleznov 1973, 1976, Zamyatkin 1980). In apomictic populations, therefore, the male nucleus eventually loses all genetic functions, although many apomicts need pollination to start the apomictic development of seed, even if fertilization does not seem to occur; it has been suggested that the attractive blossoms of certain apomictic species may have some competitive value by attracting pollinating insects from obligate insect-pollinated species in the same plant community (Faegri and Pijl 1979). Faegri and Pijl (1979) also pointed out that environmental selective pressure may in the end be decisive for the equilibrium between sexual reproduction with its diversity and asexual reproduction with its effectivity, and pollination is one of the environmental factors.

From the evolutionary point of view of breeding systems, it is inferred that the three *Fagopyrum* species, common buckwheat (*F. esculentum*), tartary buckwheat (*F. tataricum*) and perennial buckwheat (*F. cymosum*), have a common ancestor which was a homomorphic, self-incompatible, perennial form with entomophilous flowers; tartary buckwheat is phylogenetically the most ancient, and common buckwheat has arisen from perennial *F. cymosum* by divergent evolution following dispersal to different environments, particularly aided by artificial selection and domestification (Kovalenko 1986, Kovalenko and Shumnyi 1988).

Many basic floral features have been generally related to the selective power of insect pollinators over evolutionary time (Stebbins 1981). Further understanding of evolutionary pathways, especially plant-pollinator relationships and floral morphological and physiological changes in the genus *Fagopyrum* is required. There must be studies of the role of insect pollination under the influence of various natural environments and domestication in each species.

3. Pollinator-plant interactions and evolution of breeding systems in buckwheat

Most research on pollination mechanisms in buckwheat has to date proceeded in isolation from research into the evolution of breeding systems, as until very recently was the case with much other historical research of pollination ecology. However, the evolution of

breeding systems has been occuring in all species through a combination of floral traits and pollinator behavior, tightly linked to population genetics. Belated consideration must therefore be given to the genetic bases of floral adaptation for pollination and reproduction in order to expand biological and/or agronomic research for improved buckwheat cultivation.

Relating pollen and seed dispersal patterns to the genetic structure of plant populations, knowledge of potential and actual gene flow in plants was first summarized by Levin and Kerster (1974). Empirical evidence suggests that gene flow, especially through pollen dispersal, is extremely restricted in range, and typically, the pattern of insect-mediated pollen dispersal is strongly leptokurtic (Levin 1979, 1981). The relationship between pollinator movements and pollen dispersal patterns, in terms of its genetic importance, depends on such pollinator characteristics as pollen carry-over rates and directionality of successive moves between flowers (Levin 1981), as well as on such plant characteristics as the breeding system and inflorescence architecture (Wyatt 1982).

Buckwheat flowers, which generally occur in racemes but occasionally in corymbs (Shakhov 1977), each have five petaloid perianth segments, eight nectaries which alternate with the filaments of the stamens, and a superior ovary. Of the eight stamens, the inner whorl of three stamens dehisc inwards, and the ovary has three united carpels and is surmounted by three styles. Flowers occur in two forms as a distylous heteromorphic system; one morph has flowers with long styles and short stamens (pin) and the other has flowers in reciprocal positions with short styles and long stamens (thrum). The pollen grains of long-styled flowers are smaller than those of the short-styled ones.

Although self-fertilization is sometimes possible, the only compatible crosses are usually those between two morphs (Darwin 1876), so an insect probing for nectar easily becomes dusted with pollen on both sides of its body. Darwin (1877) proposed that the reciprocal positioning of anthers and stigmas in distylous taxa was a device to promote such disassortative pollination, and observed that pin and thrum pollen was found in different positions on bumblebees and moths visiting *Primula* spp..

Similarly, Rozov and Skrebtsova (1958) reported that the predominant pollens on the thoraces and abdomens of honeybees visiting buckwheat were of the long and short-styled flowers respectively; a bee usually touched the stigma of a long-styled flower with its abdomen but the stigma of short-styled one with its head and thorax.

Buckwheat flowers are abundantly visited by various insects. Honeybees are believed to be the main insect pollinator according to various reports, i.e., 63-72% of the visitors (Kopeljkievskii 1953), 86.6% (Demianowiczowa and Ruskowska 1958), etc. However, honeybees do not visit buckwheat during the first 2-3 days of its flowering.
Moreover, though honeybees are one of the important pollinators of buckwheat, they are not so good as various flies as pollinators of buckwheat cultivated in areas where many different flowers are blooming concurrently since honeybees have a strong habit of visiting buckwheat fields only at the zenith period of flowering on fine warm days, whereas various flies, such as *Eristalis cerealis* (shimahanaabu), flower and house flies, etc., are constant daily visitors during most of the flowering period, except on days of heavy rain (Namai 1986).

In terms of the relationship between the number of insect pollinators visiting a buckwheat flower and the number of pollen grains deposited on a stigma lobe of each flower in the two morphs, the % seed set which results from the intensity of so-called "legitimate pollination" between forms differing in style length, depends greatly on the frequency of visits (Namai 1986, Namai and Ohsawa 1986). Table 1 gives more detailed data on this phenomenon. The data explain the relationship between the number of insect pollinator visits to a flower and the % seed set in common buckwheat. Data were

obtained from one inflorescence in a buckwheat field and the number of visitors was counted by an 8 mm macro-cinecamera, releasing the shutter every one second from 9 to 11 o'clock in the morning for 6 days. It was concluded that three or more visitors induce a promising higher seed set percentage. On the basis of an experiment in artificially controlled pollination under an operation microscope in favorable environmental conditions, mean seed set percentage in flowers with only one compatible pollen grain deposited on a stigma lobe was about 40% and in flowers with 3 or 5 being about 70%, while flowers with 10 or more compatible pollen grains gave up to 80-90% seed set (Namai and Ohsawa 1986).

Table 1. Relationship between the number of insect pollinators visiting a flower and % seed set in common buckwheat

No. of visitors	No. of flowers observed	No. of seeds harvested	mean % seed set
0	8	2	25
1 - 2	5	2	40
3 - 4	8	7	87
5 - 6	6	6	100
7 - 8	5	5	100

Heterostyly is generally assumed to have arisen independently many times in different phylogenic lines of the angiosperms (Nettancourt 1977). Ganders (1979) considered the evolution of heterostyly an unsolved mystery. However, on the basis of many previous papers, Wyatt (1983) summarized the possible evolutionary history of the origin of heteromorphic (distylous) from monomorphic (hermaphroidite) forms and dioecy from heteromorphic forms as follows:

Stage 1 - the original monomorphic population consisting of two mating types between the heterozygous (*Ss*) and recessive homozygous (*ss*),

Stage 2 - the mating types have become dimorphic in style and stamen length, enhancing disassortative pollination and resulting in typical distyly, with the short-styled thrums being heterozygous (*Ss*),

Stage 3 - the original dioecy has become established, with the thrums representing the heterogametic male flowers and the pins homogametic female flowers.

Incompatibility reactions and morphological differences in distylous taxa are in general genetically controlled by a supergene that behaves as a simple Mendelian factor (Lewis 1949, Dowrick 1956, Charlesworth and Charlesworth 1979); the short-styled thrum is usually the *Ss* heterozygous, and the long-styled pin is the *ss* recessive homozygote (Vuilleumier 1967, Ganders 1979). A wide range of selfing and outcrossing mechanisms in diverse angiosperm taxa has been discovered by many researchers (Mather 1943, Stebbins 1950, Grant 1975, Barrett 1988). This wide diversity has led to the concept of so-called "balanced breeding systems" in plants. At present, it is generally accepted that the advantages of autogamous reproduction in terms of adaptation to the immediate environment provided by increased homozygosity are a trade off against those of outcrossing, which retains genetic variability, together with long-term evolutionary flexibility.

Morris (1951) demonstrated that in compatible matings the pollen tubes reached the base of the styles within 15 minutes after pollination, but that in incompatible combinations the growth of the tubes was checked at the base of the stigma in the short style and in the lower half of the long style; in an interspecific cross between common buckwheat and *F. tataricum*, the pollen tubes of the former reached the base of the styles of the latter within 20 minutes; the growth of the pollen tubes of *F. tataricum*, on the other hand, was inhibited in the styles of common buckwheat; the behaviour of the pollen tubes of *F. tataricum* in the long and short styles of common buckwheat was identical with that of the behaviour of the

pollen tubes in incompatible matings within common buckwheat; no hybrids from the two species were obtained. Sharma and Boyes (1961) clarified the genetic system of self-incompatibility in buckwheat in accordance with Dowrick's hypothesis of the control of incompatibility by the supergene *S* in *Primula*.

They postulated a similar gene in *Fagopyrum esculentum*, thrum plants (*Ss*) being heterozygous for the subgenes *G* (short style), *Is* (thrum incompatibility reaction of the style), *Ip* (thrum incompatibility reaction of the pollen), *P* (large pollen grains) and *A* (long thrum stamens), and pin plants (*ss*) recessively homozygous for the subgene *s*. They also described two artificially induced segregants of thrum plants (plant A and plant B), which each bore a branch with modified flowers, among plants from buckwheat fruits treated with X-rays or termal neutrons, and discussed the origin of the induced modified flowers as follows:

Plant A had a branch with homostyled flowers, differing from the thrums on the same plant only in possesing long styles, and plant B had a branch bearing both pins and thrums as well as one half-pin, half-thrum flower with four short and four long stamens and one long style and two short ones. According to the results of crossing with a control pin plant as male, they suggested that the incompatibility reactions of the pollen and style in the homostyled flowers were the same as those of the thrums on A plant, and the homostyled flowers may have originated due to (1) mutation or conversion of *G* to *g*, (2) a very small deletion or (3) somatic crossing over within locus *S*. According to the results of illegitimate crosses between flowers on B plant and legitimate crosses of flowers on this plant with pin and thrum controls, the incompatibility reaction of pollen and style in the pins and thrums of B were as would be expected for the two types of flowers on normal plants. Mating within the single half-pin flower was fertile. A thrum plant (T) in the progeny of B was notable for its relatively high degree of self fertility (28.6%), but apparently this self fertility was not a consequence of a breakdown in the incompatibility system, since pollen and stylar reactions in matings with pin and thrum controls were unchanged. The self fertility of T raised difficulties in explaining the origin of the modified branch of B according to the hypothesis previously given; possibly other genes acting on locus *S* were modified by the radiation treatment.

Zheleznov (1967) observed that a buckwheat population contained an average of 20% of individuals with some degree of self fertility, and self fertility in buckwheat is heritable but influenced by environment. Marshall (1969) described that a new flower form with shorter styles had been found and self-fertile homomorphic lines of buckwheat subsequently developed, some of which were especially adapted to self pollination since the flowers had equal pistil and stamen height, and there was a significant positive correlation between the self fertility of the parents and that of the progeny in the S_2 to S_4 generations. Khotyleva et al. (1977) reported that crosses of 16 cross combinations were made between buckwheat varieties and the F_1-F_2 hybrids were selfed; the F_1 hybrids had a higher degree of self compatibility than their parents, while F_2 was inferior to F_1 in the % seed set after selfing. It was therefore concluded that inbreeding leads to an increase in self-incompatibility, and cross pollination to an increase in self-compatible heterozygotes; an optimum level of heterozygosis is maintained in this way.

Anokhina (1979) reported that heterozygous forms of buckwheat often had the highest degree of self compatibility and crossing varieties of different origin increased self compatibility; the hybrids differed in reaction to controlled selfing. In addition, the number of inflorescences in inbred F_1 was equal to that of the more vigorous parent in only one case out of 16 and in the remaining 15 crosses the number was intermediate or lower than in either parent. Fesenko (1979) described that four homostylous forms, with short style and stamens, S_1S_2 and S_2S_2, or with long style

and stamens, *ll* and *LL*, were crossed *inter se* in a full diallel scheme and also with heterostylous plants, *sL* and *Ls*, which had the recessive marker character determinate habit; all the legitimate crosses were compatible; of the illegitimate crosses, some were compatible and some incompatible; almost all crosses of the type *SxL*, apart from the illegitimate combination *sLxsL*, proved compatible; the crosses *sLxll* and *sLxLL* were compatible; in crosses with heterostylous forms, it was best to use homostylous forms as the pollen parent, since they are able to self pollinate. Fesenko (1987) also reported that when the F_1s (with short style and long stamens) which were obtained from plants with a long style and short stamens (a dominant character) pollinated by a plant with a long style and short stamens (recessive), were selfed, the progeny from one hybrid showed no segregation (all plants had short styles and long stamen); progeny tests suggested that no selective fertilization effects were at work; it was thought that a recessive lethal factor closely linked with the recessive allele at the heterostyly locus might account for the elimination of recessive homozygotes after fertilization.

Alekseeva (1966) discovered two buckwheat plants showing different forms of male sterility in buckwheat; one of them was deficient in chlorophyll and the other had an excess. The former produced some seed on open pollination, on pollination with other lines or with other sterile types. In the latter, plants differed in the amount of seed they produced and in the pollinators that gave the best result, some giving no seed at all. A certain number of sterile plants of the same type occured in the progeny, showing the presence of both restorers and fertility maintainers. Zheleznov and Yetsenko (1970) reported that male sterility was evidently determined not only by nuclear genes, but also by the interaction of these with sterile cytoplasm; some pollinators were sterility maintainers and restorers; a scheme has been developed for detecting sterility maintainers.

Wyatt (1983) reported a positive correlation between mean precipitation during the growing season and the percentage of plants spontaneously setting fruit in the absence of pollinators in nine populations of *Arenaria uniflora* in the southern United States. He believed that autogamy arose in response to competition for pollinators (Levin 1972) between outcrossing morphs of *A. uniflora* and a second winter annual species endemic to rock outcrops, *A. glabra*, which is pollinated by the same species of bees and flies. In the varieties of rapeseed (*Brassica napus* L.) in the world, a negative correlation has been defined between the visit frequency of insect pollinators and self fertility with automatic self-pollination ability (Namai and Ohsawa 1987).

Only a few studies have been carried out in which explanations of the evolution of breeding systems with special reference to floral morphology have been examined critically in different species. It is, however, suggested that in the *Fagopyrum* species, a number of dynamic evolutions of breeding systems in connection with pollinator-plant interactions under various environmental conditions are likely to have occurred and to be occurring.

4. Male competition, female choice, and sexual selection in buckwheat

It has largely been ignored until recently that sexual selection generally occurs in plants. Stigmas on plants are often smeared with far more pollen grains than is needed to fertilize all the ovules (Haldane 1932). The existence of sexual selection in plants was first suggested by Bateman (1948). He demonstrated that sexual selection exists in plants as a result of the size difference in male and female gametes, and male competition should lead to increased production of pollen. Several papers have recently been published which consider the role of sexual selection in plants on the evolution of breeding systems and reproductive strategies.

Stephensen and Bertin (1983) defined sexual

selection as the differential reproductive success of individuals of the same sex and species that survive to reproductive age and are physically capable of reproduction as an aspect of Darwin's natural selection. Sexual selection is divided basically into male competition and female choice, which has the potential for being a potent evolutionary force in plant populations.

Male competition is divided into two categories, pre- and post-pollination competition. Plant characteristics that influence pre-pollination competition in particular are divided into the following four categories: 1. phenology, 2. flower number and arrangement, 3. proximate attractants, and 4. floral rewards. Competition should increase from predominantly selfing to predominantly outcrossing species. Cruden (1977) suggested that pollen/ovule ratios are significantly greater in xenogamous than in highly autogamous species. When the number of viable pollen grains deposited on a stigma exceeds the number of ovules that the maternal sporophyte can nourish into mature seeds, male competition as post-pollination competition will occur. Post-pollination phenomena such as 1. differential germination of pollen, 2. growth of pollen tubes, 3. fusion with egg nuclei, and 4. ovule and ovary development are potentially influenced by both the pollen deposited on the stigmas and the maternal sporophyte. Mulcahy (1971) demonstrated that pollen tube growth rates in maize varied among individuals and were associated with pollen germination ability *in vitro*, and a reasonably good correlation between *in vitro* and *in vivo* results was found in various cases (Sari-Gorla et al. 1975).

If pollen grains produced by various donors differ in genetic quality and if reproduction is not pollen limited, the maternal sporophyte is under selective pressure to allow only those pollen grains with the highest genetic quality to sire her seed crop (Janzen 1977, Charnov 1979). Female choice could operate at any of several times as follows: 1. selective pollination, 2. selective fertilization, and 3. zygotic female choice (seed and fruit abortion). Selective fertilization, seed abortion and fruit abortion shield a plant from some of the random events associated with pollination (Mulcahy 1979). Pre- and post- fertilization mechanisms of female choice should provide different advantages and disadvantages to the maternal sporophyte. It is generally estimated that the earlier the selection, the fewer the resources that are wasted, hence sporophytic incompatibility which occurs on the stigmatic surface would be the least expensive. Some of the later forms of rejection which occur in stylar tissue, ovules, enlarging seeds and/or enlarging ovaries may be based on characteristics more closely related to offspring quality. As a result of the importance of post-pollination female choice and its demonstrated effect of increasing components of offspring quality, selection will provide female sporophytes with the possibility of receiving many pollen grains in order to maximize the possibility for such choice (Mulcahy et al. 1975, 1978, Bertin 1982, Bookman 1984). Fruit abortion as a post-fertilization mechanism of female choice allows plants to respond to uncertainties associated with the number of pollinated flowers, fruit and seed predation, and fluctations in the resources available for reproduction, such as those caused by leaf herbivory (Stephenson 1981). In several species fruits are generally more likely to mature from the first rather than later pollinated flowers. This pattern of selective maturation tends to minimize the resources wasted by fruit abortion because those fruits that have received the greatest resource investment are presented (Stephenson 1981). It is also suggested that the degree of self- and cross-fertilization influences the intensity of sexual selection by affecting the variance in the reproductive output of the two sexual functions of hermaphrodites (Willson 1979, 1982).

In spite of the value of research into the pollination biology of buckwheat for practical improvement to breeding and cultivation methods, little information about it is so far

available.

In short-styled flowers of buckwheat the number of pollen grains per pollen sac ranged from 24 to 44 with the mode at 32 (128 per anther), while in long-styled flowers the number ranged from 23 to 68 with the mode at 48 (192 per anther) (Doida 1959). Primak (1973, 1976) reported that a comparison of homostylous with heterostylous forms of a buckwheat variety showed that variation in pollen grain size was greatest in short-styled homostylous forms and least in short-styled heterostylous forms. Short-styled, long-styled and homostylous forms also all have radially symmetrical single pollen grains of elongate ellipsoid shape; short-styled plants have the largest, best-filled pollen grains, their grains being almost twice the size of those in long-styled and homostylous ones. On the other hand, Pausheva (1960) suggested that the size of pollen grains and fertility ability of the pollen of a variety depend on the position of the flowers on the plant, and the largest pollen with the highest degree of fertility came from inflorescences at the base of the main stem, especially from the central anthers of the flowers which opened first.

In terms of sexual selection such as male competition and female choice in buckwheat, Petelina (1949) described how two new buckwheat varieties, 'Ivanovskaja' and 'Aleksandrovskaja', were selected at the Aleksandrovskaja State Breeding Station, the former being obtained by selective fertilization of three improved local varieties. When the two new varieties were grown mixed, their productivity was greater than when they were sown individually. This was explained by the stimulationg effect of foreign pollen. Musiiko (1949) described the application of the method of so-called supplimentary fertilization, based on the selective fertilization principles of Lysenko and Michurin, to breeding and seed growth of buckwheat etc.; in practice, the measure consisted of encouraging open cross pollination in the field; supplementary fertilization increased the yields of crops when repeated twice or three times during the flowering period and resulted in a marked reduction of empty seed; the heritable properties of the seed, particularly its resistance to diseases, were also improved. Kopeljkievskii (1949) also described the family 2/44 of 'Bogatyrj' (Hero), which was obtained by selective fertilization, as being most productive without control of cross pollination with other geographically distant varieties grown as neighbours. Krutenko (1958) reported that mechanical mixtures of seed of the same variety but obtained from crops grown under varied environments gave rise to more productive progeny than that of the highest yielding component in experiments at Odessa. Nemlienko (1969) described how open pollination of maternal plants was found to be the most useful method of studying the effectiveness of heterosis in buckwheat; heterosis appeared to be increased when a locally adapted variety was used as female parent and a variety from another region as male parent.

Zamyatkin (1967) reported that pollination with a pollen mixture from several plants led to greater set and more intensive fruit growth than pollination with pollen from only one plant; the difference was particularly noticeable in intervarietal pollination; when compared in similar conditions 51% of *Fagopyrum tataricum* flowers set fruit but only 15% of those of *F. esculentum*. Elagin (1971) also described how the most favourable type of pollination was legitimate, in which biologically compatible gametes are combined and high yields are made possible; legitimate pollination can be intensified by the use of suitable culture techniques and, in particular, by bee pollination; pollination was effected most actively in the morning, when there was an abundance of pollen from the lower inflorescences of the main stem and from the flowers which had opened first.

Skrebtsova et al. (1974) described a study of the effect of the number of visits by bees to long-styled flowers on seed set and 1000-seed weight in common buckwheat; as the number of visits increased from two to ten, there was a rise in the 1000-seed weight

in the year of pollination and in the F_1 and F_2 progeny, and short-styled forms had a higher 1000-seed weight in F_1 and F_2 than long-styled forms, owing to short-styled flowers secreting more sugar than long-styled ones. It is assumed that those phenomena are strongly connected with sexual selection altering the genetic composition in buckwheat populations.

In relation to polycross and synthetic varieties of buckwheat, Palilov and Samusenka (1970) and Palilov (1971) suggested that the F_2 from hybrid seeds which had set on short- and long-styled components of a variety grown in a polycross was superior in seed production to progeny of the same hybrid obtained from the short-styled or long-styled components of the initial population, grown separately; the degree of heterosis of seed yield in intervarietal polycross hybrids varied with the conditions of cultivation of the F_1 and with soil and climatic conditions in the year of the polycross. Differences in conditions in different years also effected the composition of the seed-progeny population. I consider that heterosis in buckwheat might be influenced both by the action on the phenotype of the hybrids and also through population genetic processes, and most cases of these phenomena resulted from such sexual selection as pre- and post-pollination competition.

Taranenko et al. (1981) reported that when inbred material selected for high self fertility and having a recessive marker character (long style) was used as the maternal parent in uncontrolled crosses with a short-styled tester, then the degree of cross fertilization varied but was always less than that of self fertilization, which was 63.8-84.1%. Anokhina (1980) also pointed out that between 30 and 70.6% of self-incompatible plants were found in three monomorphic and two dimorphic varieties; among hybrids in which a monomorphic variety was seed parent and a dimorphic one pollen parent, the greatest self compatibility was found in the heterozygotes. Fesenko and Antonov (1981) established a method of producing synthetic varieties in which 300-500 of the best plants of the breeding material were selected for yield and grain quality in the first year, inbred in the second, subjected to test crosses by the topcross method in the third and tested for yield in F_1 in years 4-6, the 4-10 best families then multiplied and tested in generations 1-3 in years 8-10 respectively; state variety trials then occupied years 11-13; plants might be selected from the first or second generation for a second cycle of recurrent selection; this breeding program is said to increase yield by 15-20%.

Shakhov (1988) pointed out that the degree of cross pollination depended on the spatial arrangement of the crossing components and the proportions of the parental plants, that it had a localized character and was affected by the genotype of the crossing components and by environmental factors; in a 3-component mixture with various proportions of plants of different forms, one or other of the pollinators was found to be given preference in cross pollination.

Successive selection of segregating populations for better performance under different environmental conditions, which is called "seasonal and/or local disruptive selection", is known to be a very valuable breeding method for improving the range of adaptability of autogamous crops (Oka 1975, Tsai et al. 1970), but very few studies have been done on allogamous crops. On the basis of the experimental result of successive cultivations of a summer type and a late-summer (autumn) type variety of buckwheat in two different cropping seasons for two years under natural selection, Namai (1983) assumed that seasonal and/or local disruptive selection would be effective for breeding widely adapted varieties of buckwheat. In Japan there are usually two cropping seasons for buckwheat cultivation: summer cropping from middle spring to middle summer and autumn cropping from late summer to late autumn. Successive cultivation of a summer and an autumn type variety was done in the summer and autumn seasons for two years. Fig. 1 shows the seed

yield variation of their progenies in the summer season of the third year. Seed yield of the summer type variety tended to rise in the strain with successive cultivation in the autumn season as did the autumn type variety with that in the spring season. In allogamous crops, successive selection under different environments every 2-3 cropping seasons seemed to be most effective. The result of the experiment might be obtained from such successive selection pressure as sexual selection and assortative mating under different environmental conditions. Sexual selection is a basic phenomenon of sexual reproduction, and a most important research item of pollination ecology and plant population structure.

Namai and Ohsawa (1986, 1988) proposed a new concept of "reproductive success rate" (RSR) pertaining to the pollen grains deposited

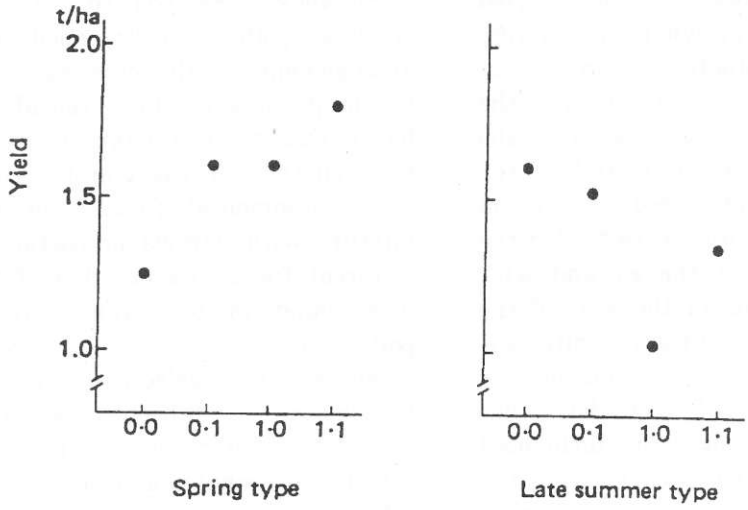

Fig. 1: Seed yield variation of common buckwheat with successive cultivation in two cropping seasons (results of third year summer season) (Namai 1983). Horizontal axis shows the cropping seasons of the last and the previous year; 0, summer season; 1, autumn season.

on a stigma (RSR-P) and the ovules in a pollinated flower (RSR-O), as well as the total intensity of transferring progenitor genes (Tr) and the intensity of genotypic variation in the progeny (Gv) on the basis of RSR-P and RSR-O, throughout studies of the pollination biology of brassica crops etc. They are calculated from the following equations:

$$\text{RSR-P (\%)} = \text{(Number of seeds obtained)} \times \text{(Number of pollen grains deposited)}$$

$$\text{RSR-O (\%)} = \text{(Number of seeds obtained)} \times \text{(Number of ovules in pollinated flower)}$$

$$\text{Tr (\%)} = \frac{2 \times \text{(Number of seeds obtained)}}{\text{(Number of pollen grains deposited)} + \text{(Number of ovules in flower)}} \times 100$$

$$\text{Gv} = \text{(RSR-P)} \times \text{(RSR-O)}$$

Tr corresponds to the possibility of seed setting. Gv indicates the ratio of the number of practical fertilizing gamete combinations producing seeds to the total number of possible fertilitzing combinations between the pollen grains deposited and the ovules in the pollinated flowers. Table 2 shows the actual RSR-P and RSR-O in pollination with different numbers of pollen grains on a stigma and the corresponding Tr and Gv values in buckwheat on the premise that there were 300 compatible pollen grains and flowers, calculated from the original data (Namai and Ohsawa 1986).

Our studies on RSR-P and RSR-O clearly reveal a route for expanding genetic variation through such male competition and female choice by limited pollination; (1) the highest RSR-P should be achieved under limited pollination with about 1:1 pollen to ovule ratio, (2) the lowest intensity of male competition in post pollination should correspond to the highest RSR-P, (3) the highest total intensity of transferring progenitor genes (Tr) should correspond to the highest total intensity of RSR-P and RSR-O in the pollinations, and (4) the widest genetic variation should occur with the highest intensity of genotypic variation (Gv).

Table 2. Variation of expected total intensity of transferring progenitor genes (Tr) and intensity of genotypic variation in progeny (Gv) in buckwheat

No. of pollen grains/stigma	No. of flowers pollinated	No. of seeds obtained	RSR-P	RSR- O	Tr	Gv
1	300	120.0	40.0	40.0	40.0	1600.0
2	150	83.1	27.7	55.4	36.9	1534.6
3	100	70.2	23.4	70.2	35.1	1642.7
5	60	41.4	13.8	69.0	23.0	952.2
10	30	26.5	8.8	88.4	16.1	777.9

5. Pollination ecology, plant population structure, and gene flow in buckwheat

Many components of a plant's life history control gene flow, microevolution and population differentiation, including generation length, cytogenetic factors such as chromosome number and crossing-over frequency, and fertilization controls such as breeding and pollination systems, population size, and external isolation mechanisms (Grant 1949, 1958, Bradshaw 1972).

Recent detailed work in pollination biology, particularly by agronomists dealing with genetically homogenous cultivated plants growing in simple environments, has shown that pollination biology, particularly the amount of cross-pollination, is very sensitive to the geometry of the population: the number of plants that are massed together, the density of flowers within the population, and the shape of the population (Handel 1983). Different sizes, densities, and shapes of populations of the same species can elicit different responses from pollinatorsand differences in the amount of pollen that is transferred. On the other hand, because natural populations have great genotypic and physiological diversity and also rarely have monomorphic pathes of plants, natural stands probably involve more complex interactions among population geometry, pollinator behavior, and gene flow (Handel 1983).

Most movements by pollinators are to very near neighbours, but occasional longer flights

also occur. Movement is controlled by their behavior, the size and pelage of their bodies, and the location of pollen grains on the insect body (Faegri and van der Pijl 1966, Proctor and Yeo 1972). When flowers on one crop species are visited by more than one insect species, different pollination effects have been seen. For example, Pedersen (1967) showed that honeybee and leaf-cutter bees visiting alfalfa gardens caused different amounts of cross-pollination and different total seed yields, the leaf-cutter bees having lower cross-pollination rates (45.4% vs 41.2%) but 19% higher yields. Although in some cases the correlation of pollinator movement patterns with actual pollen transport may be weak, pollinator movement and pollen deposition patterns have a very complex relationship (Lertzman and Gass 1983).

It has been shown that in brassica crops, pollen flow by insect pollinators is strictly dependent upon the total visiting time length on pollen parent flowers as well as the length of visit to each seed parent flower and the total visiting time on seed parent flowers (Ohsawa and Namai 1988). The correlation of cross pollination efficiency with total visiting time length on seed parent flowers was clearer with the order of flowers visited by the pollinator, and most pollen grains from the pollen parent which each pollinator (*Eristalis cerealis*, Shimahanaabu) could pollinate were deposited onto seven and more seed parent flowers for 420 sec. on average. The mean cross pollination efficiency of shimahanaabu on successive flowers was 1.3 pollen grains from different flowers per 1 sec.- visit in the case of our experiment in rapeseed. It was also shown that about 80% of pollen grains smeared on a shimahanaabu from the donor plant was depositied on only 3 to 4 flowers visited, and the main range of pollen flow by shimahanaabu was very short, up to about 1.5 m in the experimental isolation cages.

In heterostylous species in addition to *Fagopyrum esculentum*, different size classes of pollen grains are often found. Some work in natural populations of heterostylous plants in which pin and thrum colonies are spatially isolated has been done in order to study the rate of pollen collection and movement between pin and thrum flowers (Ganders 1974, Ornduff 1978, 1980), the population structure of the morphs and the rate of fruit set (Wyatt and Hellwig 1979, Wyatt 1982).

In a buckwheat population, the flight distance of shimahanaabu from flower to flower is less than 30 cm and mostly less than 10 cm, and the main radius of pollen flow by shimahanaabu is within 1 m in cages with a few pollinators, while it expands when sufficient pollinators (about 10 per 100 caged plants) are held in a cage (Namai 1986). Tables 3, 4, 5 and 6 show the other results of my study on the time course of pollination of common buckwheat in a field of Tsukuba.

As shown in Table 3, it appeared that legitimate pollination occured more frequently in thrum (short-styled) flowers than in pin (long-styled) flowers, whereas illegitimate pollination occured more frequently in pin. This assumed that the time course of the number of remaining pollen grains on an anther in Tables 4 and 5 indicated the intensity of each of the pollinators' visits. Consequently, the difference in the intensity of legitimate pollination between pin and thrum types seemed to be due to thrum flowers having some floral traits prompting insect pollinators to cause legitimate pollination even in a few visits.

As shown in Table 6, it was observed that, due to such pollination mechanisms, pin flowers mature significantly fewer seeds than thrum flowers. Skrebtsova et al. (1974) and Lee (1986) have also reported similar results in common buckwheat. A similar result has been reported in *Primula veris* (Richards and Ibrahim 1978).

Self-compatible homomorphic forms with pin and with thrum flowers of buckwheat seem to be successful even at the diploid level (Marshall 1968, 1970, Zamyatkin 1971, Fesenko and Antonov 1973, Astafjev 1974a, Antonov 1977, Kovalenko et al. 1980, Zakharov 1980, Ruszkowski and Noworolnik 1983, Kovalenko and Shimova 1986), and also at the tetraploid level (Essner 1953, Adachi et al. 1982, Dovzhenko et al. 1987, 1988,

Table 3. The time course of the maximum number of compatible pollen grains deposited on a stigma lobe of each flower in common buckwheat (n=30, mean ± standard deviation)

Flower type	Time	No. of pollen grains on a stigma lobe			Frequency distribution					
		Total	Legitimate	Illegitimate	0-5	6-10	11-15	16-20	21-30	31<
Pin	9	0.0±0.0	0.0±0.0	0.0±0.0	30					
	10	4.6±5.6	1.2±1.6	3.4±4.4	28	2				
	11	14.2±7.4	4.4±4.2	9.7±5.4	22	4	2	2		
	12	24.4±6.3	8.6±4.6	15.8±5.4	8	13	5	4		
Thrum	9	0.1±0.1	0.0±0.0	0.1±0.1	30					
	10	5.9±3.8	4.5±3.5	1.4±0.3	21	4	2	2	1	
	11	11.7±3.4	8.5±3.6	3.2±0.8	17	4	2	3	3	1
	12	19.4±8.2	14.5±5.9	4.9±2.9	4	7	7	5	6	1

30 flowers of each form were tested at each time.

Table 4. The time course of the number of remaining pollen grains on an anther of common buckwheat (n=10, mean ± standard error)

Time	Pin	Thrum
9	141.3± 6.8 (15.3)	129.4±6.9 (16.9)
10	84.7±16.9 (62.9)	85.7±6.9 (25.6)
11	36.1±10.2 (89.2)	38.0±5.8 (48.4)
12	23.5± 9.6 (129.8)	28.3±6.4 (71.8)

( ): c.v. (%). (LSD(p=0,05)=26.5)

Table 5. The correlation coefficient of the mean number of remaining pollen grains on an anther (A) with the number of pollen grains deposited on a stigma (Bs) of common buckwheat (n=10)

Between (A) and (Bs)	Time	Pin	Thrum
Between (A) and total number of pollen grains on a stigma	10	-0.792**	-0.351
	11	-0.714*	-0.534
Between (A) and total number of compatible pollen grains on a stigma	10	-0.760*	-0.395
	11	-0.612	-0.506
Between (A) and total number of incompatible pollen grains on a stigma	10	-0.790**	-0.303
	11	-0.776**	-0.536

* P < 0.05.
** P < 0.01.

Dovzhenko 1988). From the floral morphological point of view, plants with thrum flowers (short styles and stamens) tend to produce more seeds than those with pin flowers (long styles and stamens) (Adachi et al. 1982, Melkonova and Dovzhenko 1986), whereas Dovzhenko et al. (1987, 1988, 1989) and Dovzhenko (1988) obtained a self-fertile long-styled monomorphic population.

Ren and Liu (1986) studied the flying distance of various insect pollinators in large buckwheat fields, applying dye spraying. They released about 10,000 dyed pollinators and then caught by net visiting pollinators at six different flying distances from 500 to 5,000 m: among the pollinators, bumblebees had the greatest range, reaching 4,500 m, followed by honeybees that could reach 4,000 m. The

Table 6. Daily seed set percentage of pin and thrum plants from which needless new buds were properly removed in common buckwheat at open field

Flowering days	Weather in the morning			No. of plants	Mean seed set percentage	
	Weather	Mean temp.	Wind velocity		Pin	Thrum
		(°C)	(m/sec)	10	(%)	(%)
Oct. 4	fine	22.6	1.6	10	86.2 (58)	89.5 (57)
5	rain	15.1	2.0	10	16.7 (48)	32.3 (67)
6	rain	13.7	1.4	10	50.0 (40)	78.9 (57)
7	cloudy/fine	16.9	2.4	10	69.8 (53)	82.9 (35)
8	fine	18.0	2.1	10	66.7 (33)	85.4 (41)
9	cloudy/fine	17.4	2.8	10	57.7 (26)	62.5 (24)
10	cloudy/fine	18.6	1.7	5	90.5 (21)	83.3 (18)
11	cloudy/fine	21.0	2.9	5	83.3 (12)	80.0 (15)
12	cloudy/fine	20.1	1.1	5	81.8 (11)	91.7 (11)
13	fine/cloudy	17.8	1.2	5	45.5 (11)	87.5 (8)
14	fine	18.2	1.6	5	93.3 (15)	100.0 (17)

( ): Number of flowers observed.

flying ability of other pollinators was poor. They also studied pollen spreading distance by wind: 500-600 m when the velocity of the wind was less than about 3 m/sec., but up to 1,000 m when higher than 6 m/sec. It was, therefore, concluded that a safe isolation distance was about 4,000 m in areas where there are a lot of bumblebees and honeybees, and about 1,000 m when such bees are absent. Petelina (1970) considered that large-grained varieties should be tested in plots at an isolation distance of 200 m.

In order to maintain many breeding lines and the genetic resources of allogamous plant species, some simple and cheap isolation procedures are indispensable for preventing contamination. In cotton, Afzal and Khan (1950) found that some crossing occured when as much as 84 m separated fallow fields, but cotton itself was the most effective barrier; they suggested that a belt 12.2 m wide on either side of the junction of two varieties should be discarded and the remainder of the fields could be picked for seed. Simpson and Duncan (1956) also found that cotton itself was the most efficient form of isolation, and cross pollination between two varieties was reduced from 26 to 4% by a 7.6 m barrier of each variety. On the basis of our studies as mentioned above, these phenomena are easily understandable as a general feature of all crops (Namai 1986, Ohsawa and Namai 1988).

Zhebrak (1963) concluded that pollen from the 2x parent grew somewhat more rapidly in 4x pistil tissues than did pollen of the 4x parent in 2x tissues, and that pollen-tube growth was irregular in both series of crosses and increased rapidly when the temperature was raised. In some cases, the pollen-tubes ceased growth and terminal swellings appeared. The frequency of this phenomenon increased at higher temperatures. Where fertilization did occur, the development of the resultant 3x embryo and endosperm was very slow. The embryos generally degenerated at different stages of development, notably at the 4-6 day stage. He also reported that individual tetraploids grown in proximity to diploids and individual diploids grown in proximity to tetraploids failed to set any seeds in these tests. Lapinski (1968) reported that no evidence of mature triploid seed was found on tetraploid plants pollinated with haploid pollen or on diploid plants pollinated

with diploid pollen, and seed set might be reduced when diploid and tetraploid plants were grown in adjacent plots, the reduction being greater in the latter. Petelina (1974) also reported that when various diploid varieties of buckwheat were pollinated with a mixture of their own and tetraploid pollen, then all the forms selected were large-grained, early diploids with no cytological abnormalities and good resistance to cold.

According to the previous data which revealed no cross pollination effect between 2x and 4x buckwheat varieties, a tesselated plot design of 2x plants in 4x plants performed well in the USSR for isolated seed multiplication (Fig. 2). These isolation techniques are also practically applied to buckwheat breeding in Poland (Ruszkowski, personal communication). In addition, a rectangular field was more susceptible to contamination from exogenous pollen than a square one (Pedersen et al. 1969).

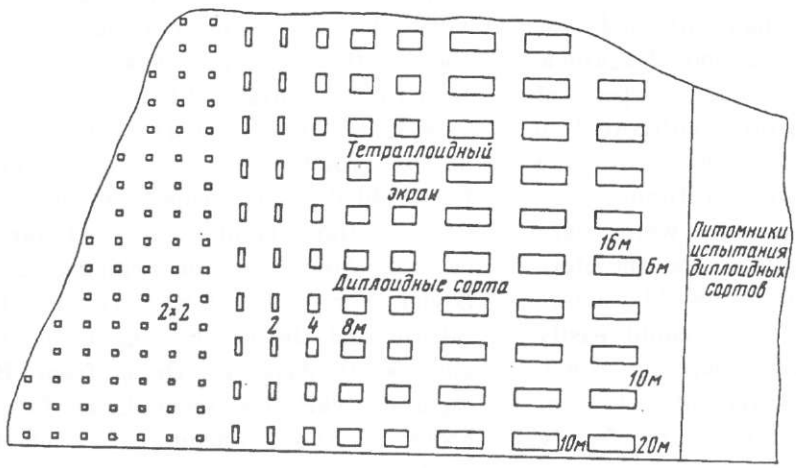

Fig. 2. Isolation field of seed multiplication in buckwheat (Fesenko 1983).

Size of plots (m^2)	Distance among plots (m)
1- 2	8
10-200	10
200 <	15 - 30

In mutation breeding of common buckwheat as an allogamous plant, a simple isolation technique is indispensable for inducing homozygous segregants for induced recessive gene(s). So spacing hill (or small row) cultures without any cages at intervals of more than 2 to 3 meters from each other is a very convenient and practical method for conducting sib-crossing within each hill (or small row) by natural insect pollinators (Namai 1989). This sib-crossing method was established through field experiments on the pollination biology of spacing hill cultures of buckwheat, arranged at various intervals from each hill with about 10 pin or thrum plants planted alternately on the basis of previous studies (Namai 1986). By means of gamma-ray irradiation (30kR) of seeds at the Institute of Radiation Breeding, NIAR, MAFF and successive sib-crossing by the simple spacing hill culture, a high yielding short-stemmed mutant was produced (Namai 1989). Basic studies concerning the reproductive biology, especially pollination ecology, plant population structure and gene flow, have great advantages in developing various breeding methods as well as in

clarifying the biological process of microevolution and population differentiation.

The following is the outline of agroecotype differentiation in common buckwheat from the evolutionary point of view, especially such pollination biology as gene flow, i.e., assortative mating which occurs easily when a population of autumn type (southern type) is cultivated under long day (more than 14.5 h) conditions.

Historically, Bazhenov (1946) pointed out that over 200 varieties from other regions and from abroad have been tested, but the ancient local race proved better than any and was chosen as the basis of selection. Nagatomo (1959) reported that summer types of Japanese buckwheat, generally cultivated in northern parts, were not so sensitive to a long-day photoperiod, whereas autumn types, cultivated in southern parts, were very sensitive; a complete autumn type therefore could not bloom in conditions of 14.5 hours day length, but summer types could easily bloom in those conditions. Krotov (1962) concluded that yield improvements of local forms were effected by mass or family selection, carried out either once or in two successive generations. Alekseeva (1967) noted that classification of local cultivars of buckwheat in the western districts of the Ukrainian SSR revealed the following ecogeographical groups: White Russian (Belorussian) (northern parts in *l.* ca. 52.5-56° N), Poles'e (subgroups: early and mid-season), forest-steppe area (Kremenec Podolskij subgroups), Dnestr valley and Carpathian (Precarpathian and High Carpathian; in *l.* ca. 48-50° N). Ujihara and Matano (1974) reported that in 26 varieties of common buckwheat collected in Japan, those from lower latitudes were taller, later flowering and had more leaves, branches and inflorescences and also that variation within characters increased as latitude decreased. Nishimaki (1975) also revealed that one of the problems encountered in the cultivation of buckwheat arises from the existence of spring (summer), winter (autumn) and intermediate ecotypes, which differ in response to day length and temperature; the summer type had early development and when sown in spring, yields of over 2 ton/ha. are fairly consistent, and if sown in summer, its growth cycle was short, growth was poor and grain yields were low; if the autumn type was sown in spring, the plant grew very tall, growth period was very long, growth was good but yields were reduced; the intermediate type had high grain yields at all dates, but excessive stem growth after spring planting and poor development after autumn planting; the yield of autumn and intermediate types fluctuated considerably with the year. The regional distribution of these different types was also described by Ujihara and Matano (1974).

Fesenko (1978) reported that the duration of the growth period in local varieties varied from 130 days in forms from India to 100-130 days in those from China and Japan, 90-100 days in those from the Primorje region of the Soviet Far East, 70-90 days in those from Siberia and the central region of the RSFSR and 60-70 days in those from Belorussia; vigorous varieties from India formed 15-20 nodes in the branching zone of the stem, while Belorussian varieties formed only 2-3 nodes; forms with the largest grain (1000-grain weight 27-33 g) occured among those from Japan, China and Belorussia (1000-grain weight 19-21 g), and the husk content in varieties from throughout the world varied from 18.5 to 26%. Gorina and Anokhina (1980) concluded that earliness was correlated with height (node) of insertion of the first inflorescence; in early varieties, the first inflorescence generally appeared at node 2-3 in Belorussia and a shorter growth period was also associated with lower yields; selection for high percentage of fully developed fruits was seen as a promising means of combining earliness with high yield. Baryura and Zakharov (1979) also concluded that the effectiveness of selection, at two different stages of development, for length of growth period, and early selection before flowering was twice as effective as selection at the end of the growth period.

Matano and Ujihara (1979) reported that

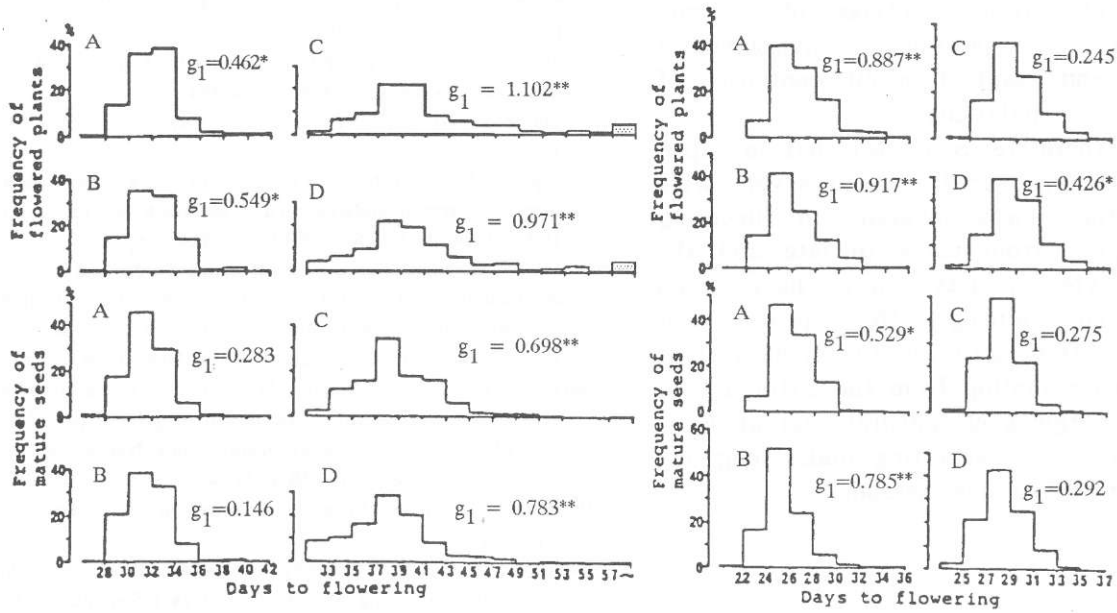

Fig. 3. Frequency distributions of total number of flowered plants and mature seeds within each flowering time of four varieties in summer cropping season (left) and autumn cropping season (right).

about 300 cultivars from all over Japan were classified by their flowering and yield responses into summer, autumn and intermediate agroecotypes; seed shape was also divided into three types, A (completely no-winged), B and C (winged), and it showed a close relationship to the agroecotypes (the seeds of most autumn types were no-winged as A but most summer types winged as C); cultivars thus classified were found to have a close relation to geographical distribution. They also suggested that the primary agroecotype might be regarded as an autumn type adapted to the climatic conditions of a low latitude, and a summer agroecotype might have originated through cultivation under different conditions. It seems that the summer type occured in northern China, northern Korea and Manchuria, and buckwheat seems to have been introduced to Japan via trade routes from Korea.

Minami and Namai (1986a, b) demonstrated experimentally that the summer ecotype may have differentiated from the autumn ecotype through natural and artificial selection during the summer in the course of its introduction from southern Japan into northern Japan; grain yield was greatly reduced in the autumn ecotype under summer cultivation; at Tsukuba (*l.* 45° N) artificial selection at 20% pressure for earliness and lateness of flowering carried out on the most typical autumn ecotype cultivar 'Miyazakizairai' usually cultivated in Miyazaki Prefecture, Kyushu Island (*l.* 31° N) was effective for late flowering in both summer and autumn cultivation seasons but selection for earliness was only effective in the summer; the highest grain yields were seen in the progeny of early flowering plants selected in the summer. They also discovered that when summer-sown plants of cultivar 'Miyazakizairai' were scored for flowering date and harvested early or late, then summer-sown progeny of the early-harvested plants tended to flower earlier than the original population, while late harvesting decreased the number of early-flowering plants.

Figure 3 gives other data obtained in Tsukuba which explained the different responses of various ecotypes of common buckwheat to seasonal environmental conditions and resulted in differentiation of their progeny populations.

It was, therefore, concluded that in Japan the autumn type variety retains a very wide potential for genetic diversity in flowering time, ranging from early to late and the summer type variety may have been differentiated during the process of introduction from southern to northern areas by assortative mating from the autumn type variety through such natural and artificial selection as early harvesting under long day condition in the summer season.

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(PBA: Plant Breeding Abstract)

Fagopyrum 10 (1990): 47-50

Research and breeding work with buckwheat in USSR (Historic survey)

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Key words: *Fagopyrum cymosum, F. esculentum, F. giganteum, F. suffruticosum, F. tataricum*, growth, pollination, scientific publications, scientific research work, yielding capacity

Buckwheat has always played a very important role in the Russian economy, at one time being produced for export. In the XIX century buckwheat was planted on more than 4 mln ha.

The first scientific publications are dated 1881 and 1889 (1, 2).

At the end of the XIXth century, buckwheat plantings were suddenly reduced. The Russian government had worked out a Programme of Research aimed at devising measures for increasing the yield of buckwheat. The scheme was elaborated by the First All-Russia Congress for Experiments in 1901. It stimulated the organization of scientific research work (3 - 8).

Some work (9, 10) was devoted to botanic classification of buckwheat.

The Genus Fagopyrum is subdivided into the species *F. esculentum* Moench, *F. tataricum* (Z.) Gaertn. *Fagopyrum cymosum Meissn., F. suffruticosum*. By crossing tetraploid forms *F. tataricum* and *Fagopyrum cymosum* the scientists A.S. Krotov and E.T. Dranenko in 1973 created a species form *F. giganteum* Krotov (11).

Russian scientists have studied many peculiarities of the biology of buckwheat. I.A. Pulman (2) established that buckwheat is a moisture-loving crop. The coefficient of transpiration of buckwheat is 2 - 3 times more than that of cereals. Buckwheat is very sensitive to moisture deficiency during the period of blossom and fruit formation.

E.A. Stoletova discovered that USSR varieties are neutral to day length, but that varieties from the Far East of the USSR, Japan, China and India develop more quickly under conditions of a short day (12).

D.N. Pryanishnikov (13) found that the buckwheat root system is able to assimilate P from hard-available compounds. It also takes up much K and is sensitive to Cl.

Many works are devoted to the biology of pollination. S.M. Korzinsky and N.A. Monteverde demonstrated Ch. Darwin's ideas of the advantages of legitime pollination (14). N.V. Fesenko (15) showed that under natural conditions, only legitime pollination is effective. Z.P. Pausheva (16) opened the phenomenon of fruit incompatibility: necrosis of legitime ovaries when legitime fruits are formed. F.E. Zamyatkin stated that necrosis takes place among fruits of legitime origin (embryon selection of ovaries of not full biological value).

Buckwheat is an indeterminant plant (18): the buckwheat flower dies off after 24 hrs without pollination, whereas other cross-pollinating plants (rye, hemp) - range from 10-20 and sometimes up to 60 days. Duration of fruit formation is limited: 21 - 24 days (19). In wheat, the range is from 20 to 40; and in corn, 35 - 63 days. This explains buckwheat's low yielding capacity.

Many investigations have been devoted to the problem of the low yielding capacity of buckwheat. This phenomenon is connected with the ovaries' dying off. Some causes for this phenomenon have been discovered:

- moisture deficiency and high temperatures at the time of blossoming and fruit formation (2);
- formation of too many flowers and ovaries and associated nutritional deficiency (20);
- imperfect heterostyllic apparatus;

insufficient pollination, deficiency of legitime pollen (21 - 23);

nutritional deficit associated with simultaneous growth, blossom and fruit formation (24).

This phenomenon has a role in the defensive-adaptation mechanisms of buckwheat. The main defensive-adaptation trait of buckwheat is assumed to be its intensive growth. Buckwheat can compete with others species owing to evolutionary adaptability to a warm and wet climate and to its capacity for long intensive growth, branching, blossoming and fruit formation. In conditions of light and moisture deficiency, nutritional elements supply not the flowers, but the vegetative organs. It causes mass dying off of ovaries, but allows the plant to grow.

The mass dying off of ovaries is thus caused by defensive-adaptation mechanisms which help the species to survive. To create buckwheat varieties with a high yielding capacity it is necessary to transform its defensive-adaptation mechanisms, which is very difficult (18).

The beginning of selection in Russia is linked to the names of I.A. Pulman (7), L.F. Althausen (8), and with the work of Shatilovskaya experimental station.

The station's first achievement was the breeding of the variety Bogatyr. The methodology of its breeding is obscure.

The work with the variety included seed sorting for grain size and weight. They have written (25) that this work was done from 1901-1909. As a result, the vegetation period of the planted variety increased by 2 weeks and yield by 78%.

The variety Bogatyr showed a high yielding capacity and ability to grow under various conditions. It has been planted in different regions of the USSR for many years. It is presented here as an example of the successful use of an evolutionary method of selection.

In the XIX century, peasants pastured herds of animals on fields intended for buckwheat planting, which is why buckwheat was planted at the beginning of June. Scientists at the Shatilovskaya station established that buckwheat should be planted during the 3rd ten-day period of May, 2 weeks earlier. In addition, the soil for buckwheat planting should be properly cultivated and fertilized.

A combination of selection of large-sized, full-kernel grain, early sowing and high yielding capacity created favourable conditions for gathering in a selected population of late-ripening, high-productive, large-sized plants and for developing a high yielding capacity.

In 1968, E.S. Alekseeva bred the variety Victoria on the basis of a local high yielding ecotype from West Ukraine.

The next stage was breeding of large-size varieties with high technological qualities of grain. All the local varieties of the USSR have small seeds with weight 20 - 22 g and satisfactory technological qualities.

The first large-size variety, Shatilovskaya 5, was bred in 1967. The donor of large size was a biotype from the variety Amurskaya mestnaya, crossed with the variety Bogatyr. Shatilovskaya 5 grain has weight 28 g; groat outcome - up to 79%. In 1971, N.N. Petelina bred an outstanding large-grain variety Krasnostreletskaya with grain weight up to 33 g. The donor of large size was a biotype of Japanese origin, from the collection material of Vavilov All-Union Research Institute of Plant Industry. On this basis, Petelina bred a large group of varieties. Varieties Shatilovskaya 5 and Krasnostreletskaya were also used for breeding large grain varieties. Small grain varieties are not used nowadays.

The breeding of tetraploid varieties was an outstanding achievement in buckwheat selection. The first tetraploids were bred in the USSR by V.V. Sakharov and A.R. Zebrak in the forties. Breeding tetraploid varieties was difficult since polyploidy caused undesirable phenomena with buckwheat (sterility, late ripening). The investigations of L.I. Dovzenko, A.I. Tkachev, E.D. Gorin, A.M. Dorofeev were devoted to avoidance of these phenomena.

The following tetraploid varieties are grown nowadays in the USSR: Bolshevik 4,

Iskra, Minchanka and others. Many scientists have tried to use heterosis for increasing the yield capacity of buckwheat. The first step in this direction was taken by G.M. Soloviev. He worked out a method of mass hybridization on the basis of heterostyly. Practical results were obtained when using a method of breeding synthetic varieties on the basis of individual-family selection for compatibility (27). The varieties Kievskaya and Orlovchanka were bred by this method.

Modern breeding might be termed mutational. It has become clear that only mutant genes can be donors of such valuable traits as limited growth, lodging resistance, narrow leafness and others.

The first success in this direction was achieved with the breeding of a determinant Sumchanka variety. The determinant form was described by E.A. Stoletova in 1940, but it was only used fo breeding purposes in 1954, by D.M. Kildishev. A number of determinant varieties have been bred to date. Their traits include: limited growth, lodging resistance and narrow leaf. These varieties give high yields in regions with a short hot summer.

Based on a form with limited shooting they have bred an ultraquick ripening variety, Skorospelaya 86, with a period from germination to ripening of 60 days. It can serve as a forecrop for winter crops.

F.E. Zamyatkin and H.G. Marshall have used homostyle autofertile forms to stimulate the breeding of autofertile varieties, which some scientists believe will revolutionise buckwheat breeding. Today, homostyly has helped to solve the problem of buckwheat inbreeding and mass selection of mutations. Several dwarf, short-stem, small-leaf and other mutants have been selected for breeding work.

The main source of initial material in the USSR is the Vavilov All-Union Research Institute of Plant Industry. It possesses a collection of more than 2000 buckwheat samples. More than 1700 of them are local varieties from the USSR.

Testing is a very important stage of the breeding process. For this purpose, the State Commission for Cultivar Testing was founded in 1937 in the USSR. It has branches for testing in all regions of our country.

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Zinc in buckwheat

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Key words: chromatography, digestion, gel filtration

Abstract

Buckwheat flour was subjected to peptic and pancreatic digestion. The soluble zinc component in the digesta was separated by gel filtration chromatography on Sephadex G-50. Approximately 72% of the zinc in buckwheat was solubilized on digestion. The majority of the soluble zinc was bound with a substance with molecular weight of approximately 1,000 dalton. Our findings suggest that zinc in buckwheat may be available for absorption.

Cink v ajdi

Proučevani sta bili peptična in pankreatska prebavljivost ajdove moke. Po tretiranju je bila topna komponenta s cinkom ločena s kromatografsko gelsko filtracijo na Sephadexu G-50. Približno 72% cinka ajdove moke je topnih na ta način. Večina topnega cinka je bila vezana na snov z molekulsko maso približno 1000 daltonov. Na osnovi ugotovitev se lahko domneva, da je cink v ajdovi moki za organizem dostopen.

Introduction

Zinc is an essential micronutrient for humans. Today nutritional deficiency of zinc is fairly common throughout the world and is one of the major nutritional problems (Prasad 1982, Sandstead 1973, Moynahan and Barnes 1973). The World Health Organization has published provisional requirements for dietary zinc (WHO 1973). A recommended dietary level for zinc has been established in several countries in recent years (IUNS 1982). Zinc is widely distributed in foods. Rich sources of dietary zinc include meat, milk, and dairy products (Welsh and Marston 1982, Spring et al. 1979, Gibson and Scythes 1982). Cereals are also a good source of dietary zinc in view of their large consumption.

Availability of dietary zinc for intestinal absorption is generally low (Hazell 1985). Furthermore, a marked variation in the absorption of zinc has been observed among different foods (Sandstead et al. 1982, Sandström et al. 1989). There are many dietary constituents, including protein, dietary fiber and phytic acid, that beneficially or adversely affect the absorption of zinc (Cousins 1985). Although the chemical form in which dietary zinc is presented to the absorptive cells appears to have a profound influence on the availability of the zinc (Sandström 1988), the chemical form in which dietary zinc occurs prior to the absorption is still not fully understood.

Buckwheat (*Fagopyrum esculentum* Moench) constitutes an important source for humans of some essential nutrients such as protein and vitamins. Although buckwheat contains zinc (Tanaka et al. 1974, Pomeranz 1983), it is unknown whether or not the zinc in buckwheat is available for gastrointestinal absorption, or is utilizable as a good source of dietary zinc.

The present study aimed to characterize the zinc present in buckwheat and then to clarify the distribution of zinc and its chemical form on the *in vitro* enzymatic digestion of buckwheat.

Materials and methods

Materials. Commercially available fresh buckwheat flour was obtained locally and used immediately. The protein content of the buckwheat flour, as determined by the micro-Kjeldahl method (N x 6.31) (AOAC 1984), was 12.49 ± 0.19 g per 100 g flour on a fresh weight basis (means ± S.D.). Pepsin (EC 3.4.23.1, from porcine stomach mucosa, 2 x cryst.) was obtained from Sigma Chemicals Co.; and pancreatin NF, from Difco Laboratories. Sephadex G-50 was a product of Pharmacia LKB Biotechnology. All other chemicals were of analytical grade.

Classification of zinc. To obtain information on the form of zinc in buckwheat flour, zinc in the flour was classified with respect to water solubility: buckwheat flour was incubated in a 20-fold volume (V/W) of water for 2 h at 37°C, followed by centrifugation at 10,000 x g for 20 min; the supernatant obtained was then assayed for zinc. Another aliquot of the supernatant was applied on a Sephadex G-50 column (1.6 x 95 cm), which had been pre-equilibrated against distilled water.

In vitro proteolytic digestion. Proteolytic digestion was performed according to the method of Akeson and Stahmann (1964) with a slight modification (Ikeda 1984). Peptic digestion was performed in 0.06N hydrochloric acid for 3h at 37°C with an enzyme-to-protein ratio of 1:100. Immediately after peptic digestion, the incubates were adjusted to pH 8.0 with 2M Tris-HCl buffer. A pancreatin solution was then added to the digestion mixtures with an enzyme-to-protein ratio 1:20 and incubated for an additional 20 h at 37°C in 0.2M Tris-HCl buffer (pH 8.0). Sodium azide was added to the digestion mixture to a final concentration of 0.025% to prevent growth of microorganisms. Immediately after digestion, the suspension was placed in an ice-cold vessel to diminish enzymatic action, and then clarified by centrifugation (10,000 x g, 20 min). An aliquot of the soluble digesta obtained was assayad for zinc. Another aliquot of the soluble digesta was applied on a Sephadex G-50 column (1.6 x 95 cm), pre-equilibrated against 0.1M Tris-HCl buffer (pH 8.0).

The insoluble fraction after digestion was incubated with 0.05 - 5.0% sodium dodecyl sulfate (SDS) solution, followed by centrifugation at 10,000 x g for 20 min. An aliquot of the supernatant obtained was assayed for zinc; and another aliquot of the supernatant was applied on Sephadex G-50 column (1.6 x 95 cm), pre-equilibrated against 0.1M Tris-HCl buffer (pH 8.0) containing 0.5% SDS.

Analytical methods. Zinc was determined with a Hitachi 208 atomic absorption spectrophotometer. In determining zinc in a solid sample, the sample was wet-ashed with sulfuric acid and 30% hydrogen peroxide prior to atomic absorption spectrophotometry. The distribution of protein in column effluents was determined by means of A_{280} measurements. Peptide content was estimated according to the method with 2,4,6-trinitrobenzenesulfonic acid (TNBS) (Goldfarb 1966), and phosphorus was assayed according to the method of Bartlett (1959).

Results

Table 1 shows the total zinc and water-soluble zinc in various foods, and the soluble zinc released on peptic and pancreatic digestion of these foods. The highest level of total zinc was found with oyster. Buckwheat flour contained a moderate amount of total zinc. The water-soluble zinc in the foods was in a range from 3 to 45% with an average of approximately 20%. Buckwheat was found to have a relatively high proportion of water-soluble zinc in relation to total zinc (Table 1). Figure 1 shows the chromatographic elution profile of the water extract of buckwheat flour on Sephadex G-50. Most of the water-soluble zinc emerged as a single peak with molecular weight of approximately 1,000 dalton.

Considerable amounts of zinc in all the

Table 1. Total and water-soluble zinc in several foods and zinc released on *in vitro* digestion of the foods.

foods	total zinc in 100 g food[1] (mg)	water-soluble zinc[1] (% total zinc)	soluble zinc in the digesta[1] (% total zinc)
buckwheat flour	2.61 ± 0.18	40.3 ± 5.8	72.2 ± 3.0
oyster[2]	36.63 ± 11.39	14.7 ± 0.6	53.2 ± 9.0
parmesan cheese[2]	4.28 ± 0.56	17.1 ± 1.6	91.0 ± 7.8
soybean meal[2]	2.61 ± 0.17	44.8 ± 1.5	40.6 ± 12.4
egg yolk[2]	3.06 ± 0.47	2.9 ± 0.7	98.3 ± 1.9
polished rice[2]	0.31 ± 0.04	18.1 ± 4.2	58.1 ± 4.9
beef meat[2]	3.83 ± 0.55	2.9 ± 0.5	48.4 ± 7.0

1. Values are means ± S.D. (n=5).
2. Data, except for buckwheat flour, is cited from reference (Ikeda 1990).

foods examined, except for soybean meal, were released in their digesta on peptic and pancreatic digestion (Table 1). A relatively high level of soluble zinc was found in buckwheat flour. Figure 2 shows the classification with respect to solubility of the zinc released on the peptic and pancreatic digestion of buckwheat flour. Analysis of the insoluble zinc on digestion indicated that 70.7 ± 4.9% (means ± S.D.) of the insoluble zinc was solubilized with SDS solution.

Figure 3 shows the chromatographic elution profile on Sephadex G-50 of the soluble digesta which occured on the peptic and pancreatic digestion of buckwheat flour. Zinc present in the soluble digesta of buckwheat flour emerged as a single peak, designated as the zinc fraction. The molecular weight of the zinc fraction was estimated to be approximately 1,000 dalton. The ultra-violet absorption spectrum of the zinc fraction is illustrated in Fig. 4. The zinc fraction had a maximum at 270 nm and a minimum at 250 nm.

Figure 5 shows the chromatographic elution profile on Sephadex G-50 of 0.5% SDS-soluble portion of the insoluble fraction which occured on the peptic and pancreatic digestion of buckwheat flour. The SDS-soluble zinc consisted of two peak fractions: one peak fraction had a molecular weight of approximately 1,000 dalton; and the other a molecular weight of 30,000 dalton or over (Fig. 5).

Discussion

Buckwheat contained a moderate amount of dietary zinc (Table 1). Noodles made from buckwheat flour have been long popular in Japan. A common noodle dish which is made from approximately 80 g of buckwheat flour, provides about 2 mg of zinc, as estimated from the data of Table 1. This indicates that the noodle dish provides a good source of zinc. The zinc in buckwheat was highly soluble in water, in comparison to that in various foods examined (Table 1). The water-soluble zinc component consisted of a single zinc fraction with low molecular weight (Fig. 2). Noodles are cooked in boiling water before consumption. The Japanese people usually drink the hot water soak, "Soba-yu" in Japanese, after eating the noodles. It has been believed that its soak may contain nutrients (Nagatomo 1984), although the scientific rationale for such a habit has been yet not fully elucidated. In terms of the high

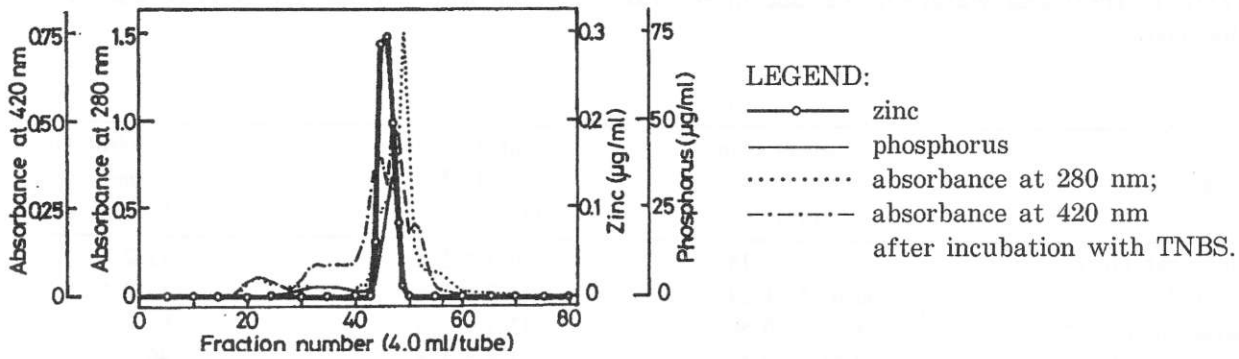

Figure 1. Chromatographic elution profile of the water extract of buckwheat flour on Sephadex G-50.

Figure 2. Classification of zinc released on digestion of buckwheat flour with respect to solubility.

water-solubility of zinc of buckwheat flour (Table 1), the hot water soak, "Soba-yu", may also be a good source of dietary zinc.

Enzymatic digestion of the foods examined, except for soybean meal, enables the amount of soluble zinc to be increased (Table 1). Buckwheat contained a high level of zinc released on digestion (Table 1), as suggested in our previous report (Ikeda et al. 1989). The zinc released was bound with a low-molecular-weight component (Fig. 3), perhaps with a protein-like substance (Figs. 3 and 4). The previous findings suggest that solubilization of dietary zinc, through its binding with low-molecular-weight components which occured on digestion, may be essential for the gastrointestinal absorption of dietary zinc (Ikeda 1990). Oyster, which contains zinc highly available for gastrointestinal absorption (O'Dell et al. 1972), is shown to consist of a soluble, low-molecular-weight zinc component on digestion (Ikeda 1990). On the

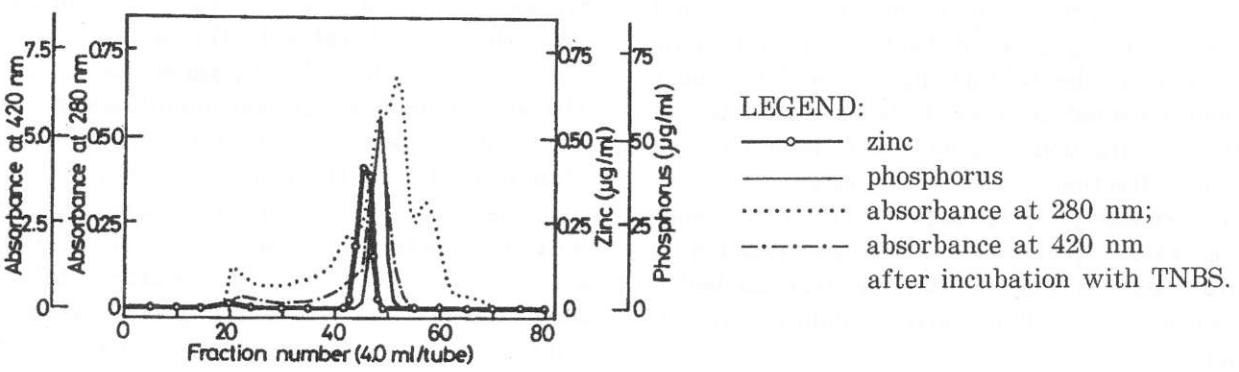

Figure 3. Chromatographic elution profile of the soluble digesta occuring on digestion of buckwheat flour on Sphadex G-50.

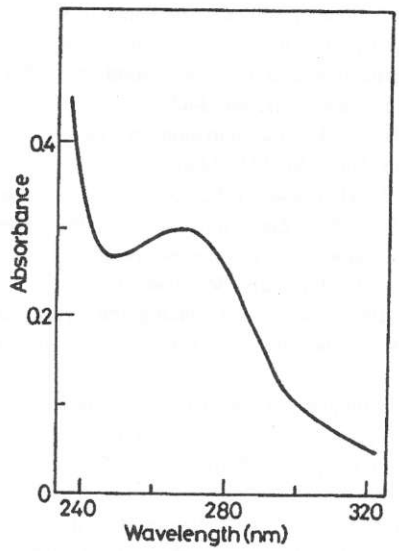

Figure 4. Ultra-violet absorption spectrum of the zinc fraction occuring on the digestion of buckwheat flour.

Absorption spectrum was determined in 0.1M Tris-HCl buffer (pH 8.0).

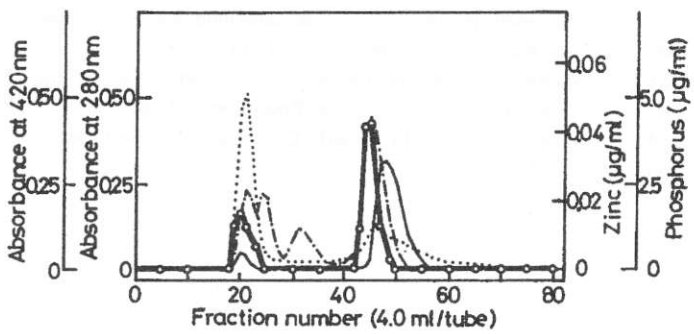

Figure 5. Chromatographic elution profile of 0.5% SDS-solution portion of the insoluble fraction occuring on the digestion of buckwheat flour on Sephadex G-50.

other hand, egg, which contained less available zinc (Oelshlegel and Brewer 1977), is shown to consist of a high-molecular-weight component on digestion (Ikeda 1990). In view of both the observed amount and chemical form of zinc on digestion, we conclude that the zinc in buckwheat may be a good dietary source and may be easily available for absorption.

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Fagopyrum 10 (1990): 57-61

Susceptibility of buckwheat (*Fagopyrum esculentum* Moench.) to *Agrobacterium tumefaciens* and *A. rhizogenes*

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Key words: *Agrobacterium tumefaciens*, *A. rhizogenes*, crown gall induction, hairy root induction, Pennquad, Siva

Abstract

The susceptibility of buckwheat (*Fagopyrum esculentum* Moench.) to tumour formation by *A. tumefaciens* and to hairy root formation by *A. rhizogenes* was investigated. Tumours were incited on stems and leaves of micropropagated plants, or on hypocotyl and cotyledon fragments of aseptically germinated seedlings. Hairy roots were also incited on stems of micropropagated plants. Plantlets and plant parts of the tetraploid cv. 'Pennquad' and the diploid cv. 'Siva' buckwheat responded very well to all *A. tumefaciens* strains. In all experiments the strain A281 exhibited a remarkable virulence, stronger than that of A348, Ach5 and A6. Hairy roots developed readily on stems, at the site of *A. rhizogenes* inoculation. Axenic tumour tissues and hairy roots were isolated from primary proliferations and cultured for more than a year on hormone-free media. It has been concluded that buckwheat is very sensitive to *Agrobacterium*, which could be used as a vector for genetic transformation.

Osetljivost heljde (*Fagopyrum esculentum* Moench.) prema *Agrobacterium tumefaciens* i *A. rhizogenes*

Ispitivana je osetljivost heljde (*Fagopyrum esculentum* Moench.) prema *Agrobacterium tumefaciens* koji obrazuje tumore na biljkama i prema *A. rhizogenes* koji obrazuje korenove ("hairy roots"). Tumori su bili inicirani na stablu i listovima biljaka koje su razmnožavane u kulturi in vitro, ili na otsečcima hipokotila i kotiledona izolovanih sa sejanaca koji su klijali u aseptičnim uslovima. Korenovi su takodje inicirani na stablu biljaka gajenih in vitro. Biljke i fragmenti biljaka tetraploidnog varieteta 'Penkvad' i diploidne 'Sive' heljde su vrlo dobro reagovali na sve sojeve *A. tumefaciens*. Soj A281 je u svim eksperimentima pokazao izuzetnu virulenciju, znatno jaču nego sojevi A348, Ach5 i A6. Korenovi su se razvili na stablu, na mestu inokulacije *A. rhizogenes*. Od primarnih proliferacija su izolovani aksenično tumorsko tkivo i korenovi, koji se gaje više od godinu dana na podlozi bez hormona. Zaključeno je da je heljda vrlo osetljiva prema infekciji koju izaziva *Agrobacterium*, koji bi prema tome mogao da se iskoristi kao vektor za genetičku transformaciju ove vrste.

Introduction

The soil bacteria *A. tumefaciens* and *A. rhizogenes* are known to induce crown gall tumours and hairy roots, respectively, by the insertion of a part of the plasmid DNA into the plant genome. Since the genes located between the ends of the transferred DNA are expressed in plant cells, plasmids carrying this DNA can be used as vectors for genetic engineering. A number of plant species has so far been successfully transformed, and

transgenic plants of several species are reported to carry new traits that improve their economic value (Fraley et al. 1986; Zambryski et al. 1989). Although most dicotyledonous plants are susceptible to *Agrobacterium*, numerous examples indicate that tumourigenesis specifically depends both on the type of plasmid harboured by the bacteria, and on the plant genotype. The intended genetic transformation of a species must, therefore, be preceded by investigation of the plants' susceptibility to the vectors. The genus *Fagopyrum* has been mentioned in recent reviews (De Cleene and De Ley 1976, 1981) as a possible host for *A. tumefaciens*, but not for *A. rhizogenes*. The objective of the present work was therefore to investigate the susceptibility of buckwheat to various common strains of these pathogens and to elaborate a procedure for possible plant transformation. This is part of a long term study aimed at introducing unconventional methods in buckwheat breeding.

Material and methods

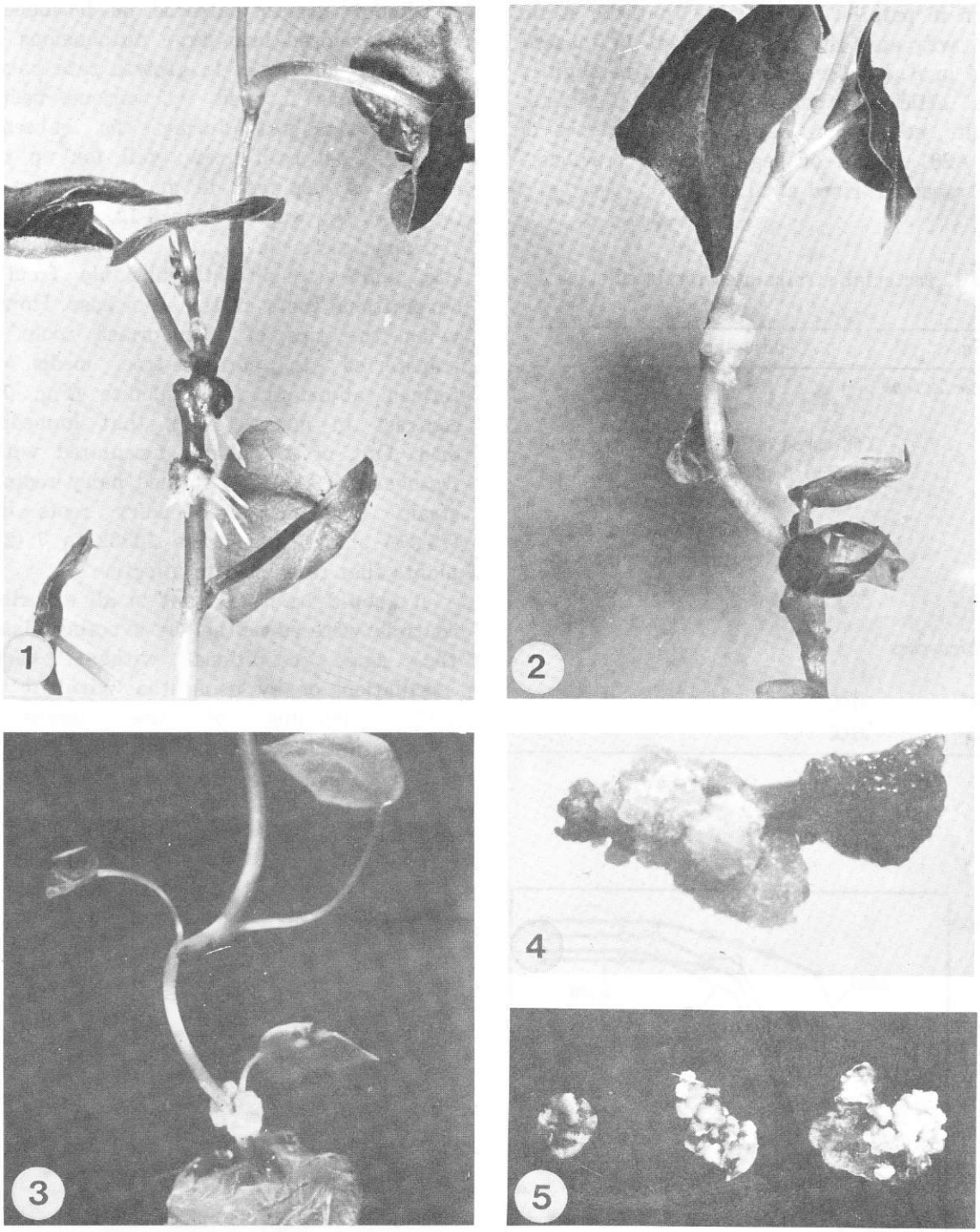
The tetraploid cv. 'Pennquad' and the diploid cv. 'Siva' of buckwheat (*Fagopyrum esculentum* Moench.) were used in the experiments. Sterile plantlets, obtained either by micropropagation or from aseptically germinated seeds were inoculated with various bacterial strains. The micropropagated clones originated from immature embryos which had developed a shoot-producing callus in culture (Nešković et al. 1987). Sterile seedlings were raised from surface sterilized seeds, planted in test tubes containing agar solidified half-strength B5 salt medium (Gamborg et al. 1968).

The bacterial strains used for inoculation are listed in Table 1. All bacteria were maintained on agar (1.5%) solidified YEB medium. For inoculation of plant material, bacteria were grown overnight at 30°C in a plate culture, or in liquid medium with shaking, to about 5 x 10^8 cells per ml.

The stems of intact micropropagated plants, about 5 cm high, were wounded by a sterile needle and a dense slurry of *A. tumefaciens* or *A. rhizogenes* cells was smeared over the wound. For *A. tumefaciens* inoculation, a variation of the leaf disk method (Horsch et al. 1985) was also employed, using cotyledon fragments and hypocotyl sections of germinated seedlings, as well as the leaf disks and petioles of micropropagated plants. Three days after inoculation, all plants and plant parts were transferred to hormone-free basal nutrient medium used for buckwheat (Srejović and Nešković 1981). Growth of *A. tumefaciens* was suppressed using carbenicillin, 1 mg ml^{-1} in the first, and 0.5 mg ml^{-1} in subsequent subcultures. For the elimination of *A. rhizogenes*, 0.5 mg ml^{-1} and 0.3 mg ml^{-1} of cefotaxime was used. When tumours developed, the proliferating tissue was excised and cultured on the same hormone-free medium. The root tips about 10 mm long that developed after *A. rhizogenes* inoculation were excised after 18 days and transferred to Petri dishes containing 10 ml of hormone-free media. All cultures were grown under white fluorescent light (5.0-7.2 $W.m^{-2}$), with 16 h light cycles, at 25±2°C.

Results and discussion

The modified leaf disk procedure produced better results in tumour induction than the inoculation of intact plants, since the plantlets had thin and delicate stems and apparently suffered from mechanical injury. Nevertheless, tumours were induced at the wound sites with all virulent bacterial strains in almost all plants that survived the treatment (Figs. 1-5). In cotyledon and hypocotyl segments, about 90% of explants responded to the inoculation by forming transformed callus tissue. The A281 strain showed the highest virulence in all experiments, followed by Ach5, A6 and A348. Hypervirulence was evident from the dynamics of tumour formation in cotyledon disks (Fig. 6) and hypocotyl segments (not presented here). After 25 days, tumours incited by the A281 strain were large (Fig. 5) and had approximately double fresh weight (about 60 mg per callus) of those induced by other strains. It was



Figs. 1-5. Tumour formation by *A. tumefaciens* on buckwheat, 3 weeks after inoculation. 1, 2 and 3: Strains A348, A281 and A6, respectively, induced tumours at the site of infection of micropropagated 'Siva' (1, 2) and 'Pennquad' (3) buckwheat plants. 4: Hypocotyl section of a 'Pennquad' seedling with tumours induced by Ach5 strain. 5: Leaf disks of an *in vitro* multiplied 'Pennquad' plant infected with (from left to right): A136 (avirulent), A348 and A281 strains.

shown in parallel experiments that the strain A281 was also the most virulent in tobacco, while in *Kalanchoe* Ach5 tended to be slightly more efficient than the other 3 strains (results not shown here). Hypervirulence of the A281 strain on legumes has previously been reported (Hood et al. 1987).

Table 1. Bacterial strains used for inoculation

Strain	Description
A. tumefaciens	
A136	C58 cured of Ti-plasmid, avirulent
A281	A136 with pTiBo542, contains succinamopine and mannopine
A348	A136 with pTiA6NC, contains octopine
Ach5	wild type, contains octopine
A6	wild type, contains octopine
A. rhizogenes	
15834	ATCC
13332	ATCC

Tumour tissues cultured on hormone-free media developed into large autonomous calli. The initial difference in growth rate between the tumours induced by various bacterial strains was lost during the subsequent passages. All calli grew well for up to 12 months as displayed in an index of growth $(W_1-W_o)/W_o$ ranging from 6-12.

Hairy roots developed at the inoculation site and were indistinguishable from the adventitious roots at the stem base. However, when the tips of transformed roots were transferred to hormone-free media, they formed abundant root colonies (Fig. 7), in contrast to normal roots that immediately died. Out of 73 plants inoculated with *A. rhizogenes*, 29 (39.7%) formed hairy roots. The strain 15834 produced hairy roots in 22 (75.8%) plants, and strain 13332 in 7 (24.1%) plants that responded to infection.

It should be noted that in all experiments controls were run either by exposing plants to the same conditions without bacterial inoculation, or by using the avirulent strain A136. Swelling of the tissues, cell

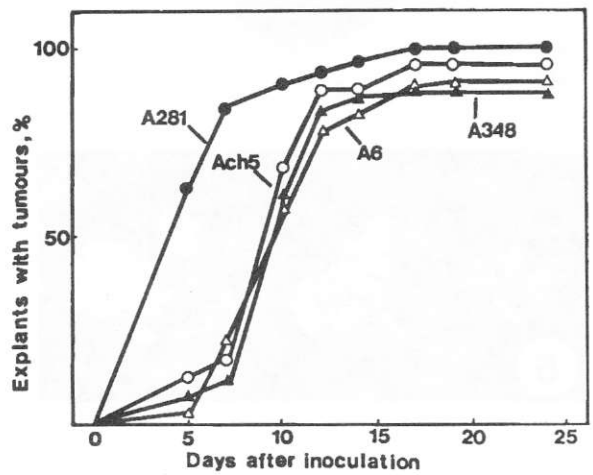


Fig. 6. Dynamics of tumour formation by various *A. tumefaciens* strains in buckwheat cotyledons; about 50 cotyledons were infected with each bacterial strain.

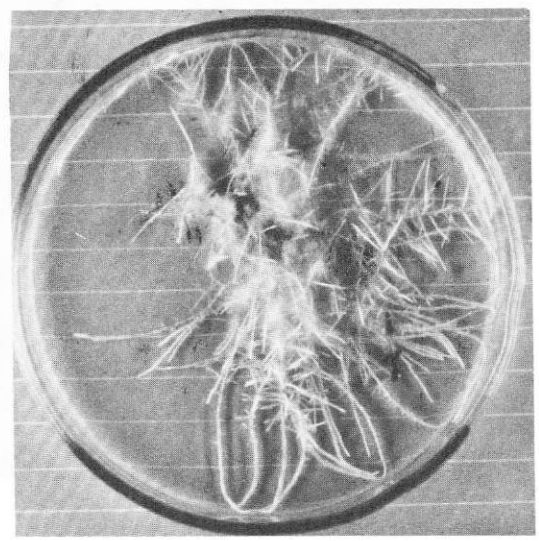


Fig. 7. Hairy roots produced by *A. rhizogenes* inoculation on a 'Siva' buckwheat stem and cultured axenically in hormone-free medium for 3 months.

proliferation, or root formation did not occur in any control samples.

The autonomous growth of axenic callus tissues and roots was taken as preliminary evidence that genetic transformation did occur. Analyses of tumour extracts by paper electrophoresis showed that tissues transformed by Ach5, A6 and A348 produced octopine, while A281-transformed tissue contained mannopine. Tumour DNA hybridized with the corresponding DNA probes in Southern blots. These results will be presented elsewhere.

In conclusion, we have shown that inoculation of buckwheat seedlings or excised organs with 4 oncogenic strains of *A. tumefaciens* resulted in tumour formation in virtually all recipients. Inoculation with *A. rhizogenes* induced the development of hairy roots. These results show that buckwheat should be added to lists of species that are susceptible to *Agrobacterium* and that projects for obtaining transgenic plants may be feasible.

Acknowledgement

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Callus regeneration from hypocotyl protoplasts of tartary buckwheat (*Fagopyrum tataricum* Gaertn.)

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Key words: *Fagopyrum tataricum*, nurse culture technique, protoplast

Abstract

Hypocotyl protoplasts of tartary buckwheat (*F. tataricum*) were cultured on either solidified or liquid modified MS medium using a nurse culture technique. Maximum plating efficiency could be raised to 25% of the living cells by means of the technique used in this experiment. Calli were obtained four weeks after protoplast isolation, but shooting could not yet be induced.

Regeneracija kalusa iz hipokotilnih protoplastov tatarske ajde (*Fagopyrum tataricum* Gaertn.)

Protoplasti, dobljeni iz hipokotilov tatarske ajde (*F. tataricum*) so bili gojeni na trdnem ali tekočem MS mediju ob uporabi tehnike sokultiviranja s kalusnim tkivom navadne ajde. Z uporabo te tehnike je bila maksimalna dosežena uspešnost iniciacije delitev celic do 25 %. Nastanek kalusa je bil opažen 4 tedne po izolaciji protoplastov, regeneracija brstov pa za zdaj še ni uspela.

Introduction

Regeneration from protoplasts is one prerequisite for the successful use of somatic hybridization. Quite recently Adachi et al. (1989) were successful in plant regeneration from protoplasts in common buckwheat (*F. esculentum*). Common buckwheat shows some excellent properties such as the extraordinary nutritional value of the grains and a short vegetative period, but its breeding faces difficulties due to heteromorphic self-incompatibility. The wild species *F. tataricum* provides genetic resources such as self-fertility and high productivity. It was therefore suggested that somatic hybridization of the two species could lead to improvements in common buckwheat varieties. In a further step towards asexual hybridization this report deals with the culture of protoplasts and the regeneration of calli in the species *F. tataricum*.

Materials and methods

Dehulled and surface sterilized seeds were germinated on 1/10 hormone free MS medium containing 1% sucrose. Pieces 1 cm in length of 7 day old etiolated hypocotyls were cut longitudinally and preplasmolysed for one hour in 0.6 M mannitol supplemented with 5 mM $CaCl_2$. The plasmolyticum was then removed and replaced by an enzyme solution containing CPW salts, 0.6 M mannitol, 1% Cellulase R10, 0.5% Macerozyme R10. The hypocotyls were incubated statically at 25°C in the dark for 16-18 hours. The protoplasts were collected by filtration through 60 µm nylon mesh and purified by washing two times with 0.6 M mannitol in 5 mM $CaCl_2$ and finally floated on 20% sucrose in 5mM $CaCl_2$. The protoplasts were initially cultured at a density of $5x10^4$ protoplasts per ml in either liquid or agarose solidified (0.5% final conc.) MS medium supplemented with 0.5 M

mannitol, 3% sucrose, 2 mg/l NAA, 1 mg/l BA and 5 mM $CaCl_2$. The pH was adjusted to 5.8 prior autoclaving. To improve cell division, cultures were activated with a nurse callus from a vigorously growing callus culture of common buckwheat. The nurse callus was either placed on the surface of the agarose plates or on an agarose drop surrounded with the protoplast suspension. The cultures were kept in the dark at 25° for the first four weeks. The osmolytic pressure of the medium was reduced after 7, 14 and 28 days with an equal volume of protoplast medium containing 0.3 M and 0.0 M mannitol, respectively. After four weeks, colonies about 0.3-0.5 mm in diameter were placed in liquid droplets or agarose blocks on top of an agarose solidified callus proliferation medium and transferred to continuous light. After two or three weeks, the growing calli were transferred to a regeneration medium (Tab. 1).

Results and discussion

Cell division started 3-5 days after activation and was slightly earlier in the bead-type like culture than in the liquid culture. Plating efficiency (PE) reached 25% in the bead-type like culture compared to 14% in the liquid culture (Table 2). Microcolonies became visible four weeks after protoplast isolation. Colony formation was 36% in the liquid culture and 28% in the bead-type like culture (Table 3). Protoplasts of *F. tataricum* generally tend to browning, particularly detrimental to growth and development in agarose plates. This could be improved by preselection of seedlings which did not dye the germination medium and additionally by frequent dilution of the liquid medium or replacement of the medium surrounding the agarose blocks in the bead-type like culture with new medium (Puonti-Kaerlas et al. 1988). Colonies doubled their size within 2-3 weeks upon transfer to callus proliferation medium and continued their growth also on the regeneration medium to form white to light-yellow calli. Because *F. tataricum* is quite reluctant to regenerate in tissue culture from various explants e.g. hypocotyls, immature embryos or axillary buds (not published) an optimal regeneration medium is still not established. A low regeneration capacity was also observed in the wild species *Fagopyrum cymosum* (Takahata 1988).

This is the first report on protoplast culture in *F. tataricum*. It could be shown that it is possible to raise the PE from approximately 1% (Adachi et al. 1989) to over 4% by means of the nurse culture technique. This was also reproducible with several common buckwheat varieties (Data not shown). Without a nurse callus, protoplasts started to divide 7-10 days after isolation independent of the culture conditions. The development stopped in the two-cell stage and after two weeks the culture collapsed. This was also observed in potato by Hein and Schnieder (1986).

Tab. 1. Media protocol for protoplast culture in *F. tataricum*

Components	Protoplast	Callus	Regeneration
MS basal medium	0	0	0
$CaCl_2$	5 mM	-	-
Sucrose	3 %	6 %	3 %
Mannitol	0.5 M	-	-
BA	1 mg/l	1 mg/l	2 mg/l
NAA	2 mg/l	1 mg/l	0.1 mg/l
Agarose	(0.5 %)	0.7 %	-
Agar	-	-	0.7 %
pH	5.8	5.8	5.8

Tab. 2. Plating efficiency (PE)* of *F. tataricum* protoplasts four weeks after culture initiation

Culture	Cells counted	Living cells	PE (%)	PE (%) of living cells
bead-type like	1000	171	4.3	25
liquid	1000	247	3.6	14

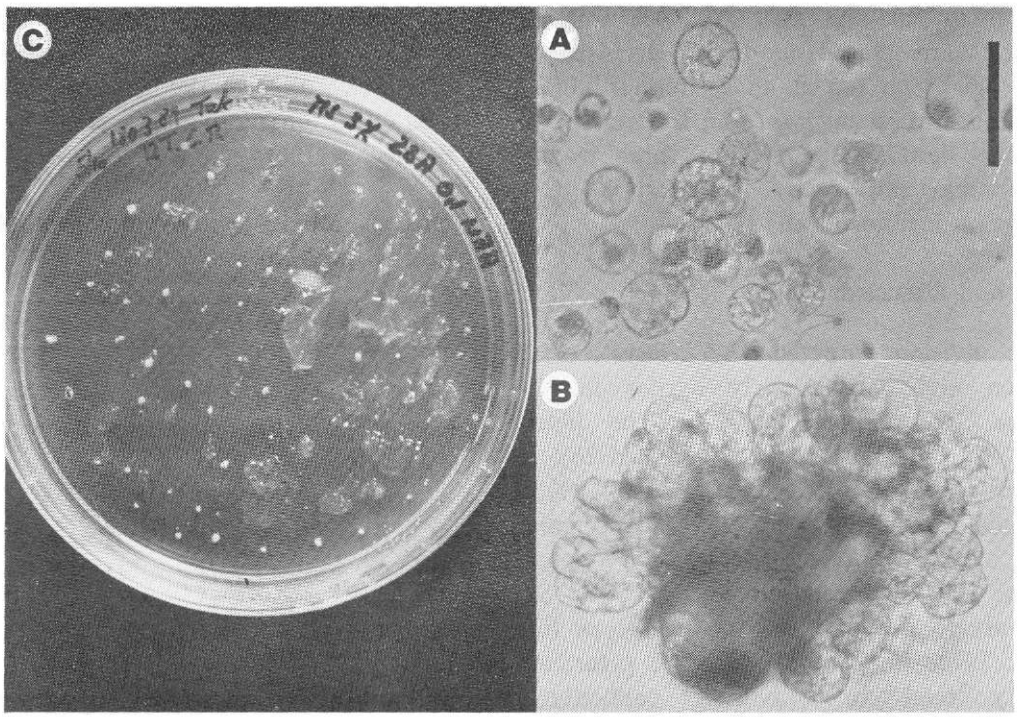
$$*PE = \frac{\text{no. of dividing cells}}{\text{total no. of cells counted}} \times 100$$

Tab. 3. Colony formation of *F. tataricum* protoplasts four weeks after culture initiation

Culture	Living cells	Dividing cells	Colonies bigger than 10 cells	Colonies (%) of dividing cells
bead-type like	171	43	12	27.9
liquid	247	36	13	36.0

This report taken in conjunction with the communication of Adachi et al. (1989) provides some evidence of the applicability of somatic hybridization in buckwheat breeding.

Fig. 1. Callus formation from protoplasts of *F. tataricum*. A. Freshly isolated protoplasts. Bar = 100 μm. B. Protoplast-derived cell colony four weeks after culture initiation. C. Calli on regeneration medium.



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Isolation and macro molecular analysis of chloroplast DNA in common buckwheat

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Key words: ctDNA, silica sol gradient, restriction endonuclease, *Fagopyrum esculentum*

Abstract

The chloroplast in common buckwheat was isolated from the leaf extracts by silica sol gradient, subsequently its DNA was purified. To analyze its macro molecule, we observed the electrophoretical patterns of DNA fragments digested with the three restriction endonucleases.

Izolacija in raziskava DNK kloroplastov pri ajdi

Kloroplasti ajde so bili izolirani iz ekstraktov listov s silika sol gradientom. Fragmenti DNK, dobljeni po tretiranju z restrikcijskimi endonukleazami so bili raziskani z elektroforezo.

Introduction

There has been an explosion of information concerning the structure, gene content and expression of the chloroplast genome in a wide range of plants in the last decade (Shinozaki and Sugiura 1986). Chloroplast is not only an important apparatus for photosynthesis, its genetic system is organized in a relatively simpler structure than that of the nuclear and mitochondria genomes (Palmer 1985). Additionally, chloroplast DNA (ctDNA) has become a valuable marker for investigating phylogenetic intra and/or inter genus relationships (Kishima et al. 1987).

However, we have no knowledge of the chloroplast genome in *Fagopyrum* species. In this report we prepared purified ctDNA from Japanese variety of common buckwheat and electrophoretical analysis after digesting with three different restriction endonucleases.

Materials and methods

Plant material: The Japanese common buckwheat variety "Miyazaki Ootsubu" was used in this study. The plants were cultivated under greenhouse conditions. Before the experiment, the plants were grown in the dark for 48hr in order to decrease starch deposition.

Preparation of ctDNA: Isolation of ctDNA was carried out essentially according to the method of Mikami et al. (1984). About 50g of leaves were homogenized in 3 volumes of buffer AMB (50mM Tris-HCl pH8.0; 7mM EDTA; 0.35M Sucrose; 5mM 2-mercaptoethanol; 0.1% BSA). After filtration through 4 layers of gauze and 2 layers of miracloth, the filtrate was centrifuged for 10 min at 10000 x g in a swing rotor. The pellet was suspended by buffer AM (lacking BSA in buffer AMB), and centrifuged. The resultants of pellet were loaded on a stepwise 20-40-60-80% Ludox AM containing 1% Ficoll, 3% PEG and 1% BSA in isolation buffer (0.35M sorbitol, 2mM EDTA, 25mM Tris-HCl pH7.5), and centrifuged at 1000 x g for 0.5hr in an angle-bucket rotor as described by Price et al. (1987). Chloroplast fraction was collected and gently diluted to 3 volumes with buffer AM, and pelleted at 1000 x g for 20 min.

The pellet was resuspended in TE buffer (50mM Tris-HCl pH8.0; 10mM EDTA), lysed by addition of 3% Sarkosyl and proteinase K (1000μg/ml). After overnight incubation, DNA was extracted from the equivalent of phenol-chloroform (1:1). DNA was purified by precipitation with 2.5 volumes of ethanol and CsCl-ethidium bromide equilibrium centrifugation as described by Sugiura and Kusuda (1979).

Restriction endonuclease analysis: HindIII, PstI and SmaI purchased from Nippon gene and Takara Shuzou were used to digest purified DNA. 1.5μg DNA was supplied in a reaction of 20μl enzyme solution. Electrophoresis was performed on 0.8% agarose gel by the method of Sugiura and Kusuda (1979). After electrophoresis, the gel was stained with ethidium bromide and photographed with 254 nm UV light.

Results and discussion

A) Chloroplast isolation: In this experiment, we chose the use of silica sol (Ludox AM) stepwise gradient to isolate chloroplast fraction rather than the sucrose stepwise gradient method which is commonly employed, since the silica sol gradient method does not require ultra centrifugation, and it is therefore convenient to manipulate and rapidly to complete the step.

As shown in Figure 1A., the chloroplast band appeared at the 20 - 40% interface in the centrifuged gradient. Microscopic observation indicated that this fraction consists primarily of intact chloroplast (Figure 1B.), and is devoid of other cell components and any debris as far as examined. Following the chloroplast lysis, ctDNA was purified by CsCl density gradient ultra centrifugation.

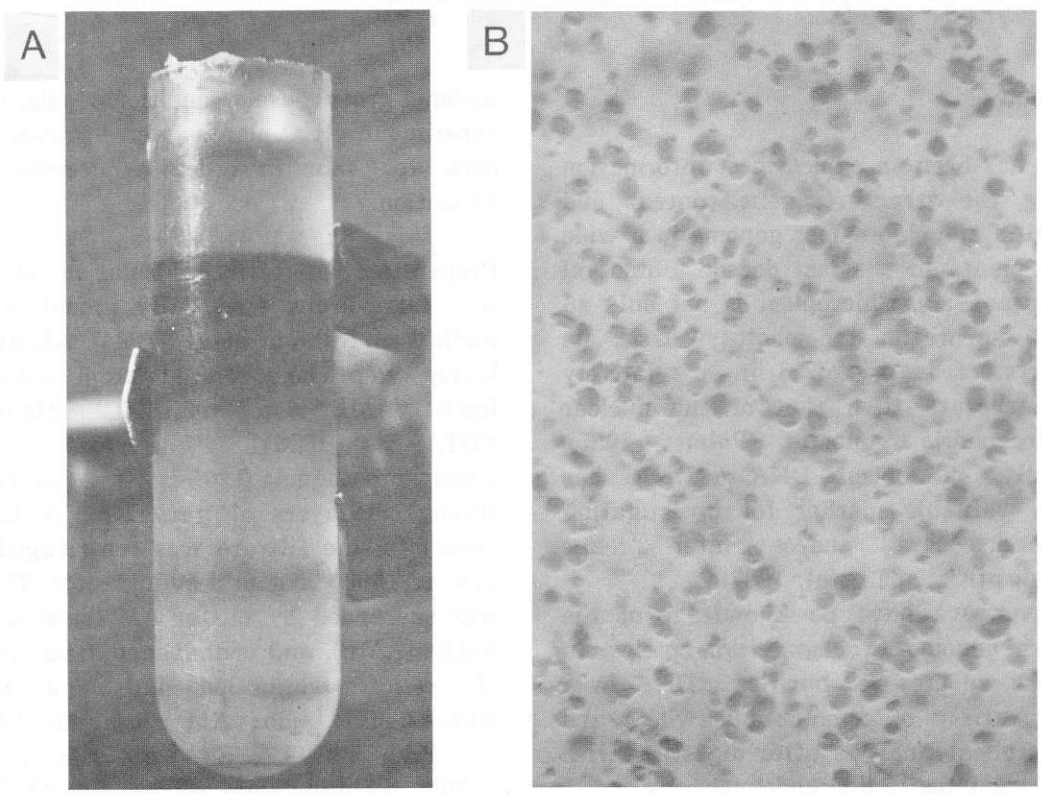

Figure 1. Isolation of buckwheat chloroplasts by using silica sol gradient. A. Centrifuged chloroplast fraction is found as thick band between 20-40% silica sol layer. B. Microscopic observation, at a magnification of 400, of the chloroplast isolated from silica sol fraction.

B) Restriction endonuclease analysis: Figure 2. represents the electrophoretic profiles of completely digested ctDNA with three different enzymes, HindIII, SmaI and PstI. Appearance of these discrete fragments revealed that the DNA sample examined here

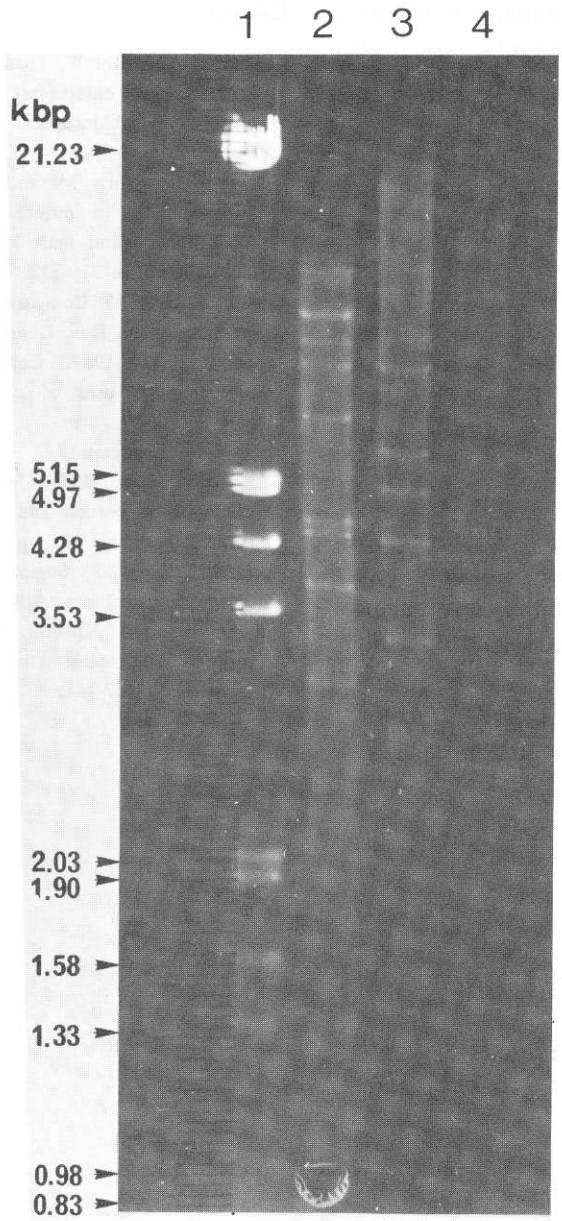

Figure 2. Restriction endonuclease fragment patterns of buckwheat ctDNA. Lane 1. HindIII and EcoRI double digest of lambda DNA; 2. buckwheat ctDNA digested with HindIII; 3. digested with SmaI; 4. digested with PstI. Numbers at the left side indicate the molecular sizes of lane 1. used as size marker.

was without contamination by other kinds of DNA molecules. When contaminated, faint bands or smear patterns can be observed.

In the case of the HindIII electrophoregram, at least 18 fragments were detected ranging in size from 16.0 to 1.03 kilobase pairs (kbp). SmaI and PstI digests from buckwheat ctDNA display 11 and 10 bands, respectively, in this profile. In Sma I and Pst I cleavage patterns, however, certain DNA fragments of high molecular size, obviously larger than 20.0 kbp, are slightly visible.

When lambda DNA treated by double digestion with HindIII and EcoRI was used as size marker,an approximate chloroplast genome size for buckwheat was estimated. By summing the molecular size of HindIII fragments, about 149 kbp was given in size. Taking into account many other species data (Palmer 1987), this value is quite plausible, although further investigation is necessary to be exact.

This is the first communication involving buckwheat chloroplast genomes. To elucidate the taxonomic and evolutionary relationships in the genus *Fagopyrum*, we are comparing the restriction endonuclease patterns by using a number of species.

Acknowledgements

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Influence of fiber on the enzymatic digestion of casein

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Key words: *Fagopyrum esculentum*, buckwheat, chymotrypsin, enzymes, pepsin, seed coat, trypsin

Abstract

The influence of dietary and acid detergent fiber with varied particle size on casein digestion by pepsin, trypsin and chymotrypsin was investigated in vitro. The highest inhibition (10-15%) was found for trypsin and chymotrypsin with the addition of acid detergent fiber.

S y n o p s i s

Badano in vitro wpływ błonnika pokarmowego i błonnika detergentowego kwaśnego o zróżnicowanych rozmiarach cząsteczek na trawienie kazeiny przez pepsynę, trypsynę i chymotrypsynę. Znacznie wyższą inhibicję (10-15 %) stwierdzono w przypadku trypsyny i chymotrypsyny z dodatkiem błonnika detergentowego kwaśnego.

R e s u m é

On a étudié in vitro l'influence de DF et ADF a différentes dimensions des molécules, sur la digéstion de la caséine par pepsine, trypsine et chymotrypsine. L'inhibition importante (10-15%) a été mis en evidence en cas de trypsine et chymotrypsine en presence de ADF.

Vpliv vlaken na encimatski razkroj kazeina

Proučevan je bil vpliv vlaken z različnimi velikostmi delcev na in vitro razkroj kazeina s pepsinom, tripsinom in himotripsinom. Najbolj je bil razkroj oviran (za 10 do 15 %) pri dodatku kislih detergentnih vlaken.

Introduction

The high content of lysin in buckwheat protein causes the nutritive value to be high compared to cereals (Amarowicz and Fornal, 1986; Javornik et al., 1981). However, experiments with animals has shown low digestibility of buckwheat protein, which may be due to the high content of fiber and tannins in the seeds (Eggum et al., 1981). Numerous in vitro experiments have demonstrated that fiber causes changes in the activity of the digestive juices in the duodenum. Electrostatic and other interactions may occur between various fiber compounds and the digestive enzymes (Hesik and Bartnikowska, 1987).

In earlier in vitro experiments (Amarowicz et al., 1988) buckwheat fiber was found significantly to reduce the amount of alpha-amino nitrogen released from cereals by trypsin and chymotrypsin. Acid detergent fiber inhibition was clearer than that of dietary fiber.

The aim of the present work was further investigation of the above phenomenon, with particular consideration of the influence of fiber particle size on the inhibition of protein proteolysis.

Material and methods

Dietary fiber (DF) was obtained from buckwheat seed coat according to Schweizer and Wursch (1979) and acid detergent fiber(ADF) according to van Soest (1963). The material was ground and separated on sieves into fractions 260-150 μm, 150-105 μm and <105 μm.

400 mg of casein (Fluka) and 20 mg pepsin (IE), trypsin or chymotrypsin (Serva) were dissolved in 25 ml 0.02 M chydrochloric acid or phosphate buffer (pH 7). Control samples were fiber free, while those examined contained the addition of 50 mg of DF and ADF which had been previously obtained. After 1 hour incubation at a temp. of 37°C combined with shaking, the enzymatic reaction was interrupted for the addition of 10 ml of 5% trichloroacetic acid. After filtration, the tyrosine content in the solution was determined by reaction with Folin-Ciocalteau's reagent (Mejbaum-Katzenllenbogen and Mochnacka, 1966). Each sample was tested 4 times. The mean values obtained were compared using the t-Student test.

Results and discussion

The results obtained are presented in Tables 1 and 2. The amount of tyrosine released from casein by pepsin diminished with the addition of DF (fractions 150-105 μm and <105 μm) and of ADF (fraction 260-150 μm) (Table 1). Using trypsin, the inhibitory effect was observed with the addition of DF fraction 260-150 μm and with the addition of all three ADF fractions. The amount of tyrosin after enzymatic hydrolysis with chymotrypsin was obviously lower than on the addition of both kinds of fiber, regardless of particle size.

Table 1. Influence of the addition of fiber on the amount of tyrosine (μmol) released by enzymes under experimental conditions.

Sample		Pepsin	Trypsin	Chymotrypsin
Control		39.1±0.3	33.7±0.5	45.1±0.2
With the addition of DF				
Fractions:	260-150 μm	38.7±0.8(C**)	31.8±0.7(A**)	42.1±0.9(A**B**C**)
	150-105 μm	36.5±0.5(A**B**C**)	32.8±0.7(B*)	44.2±0.4(A**B**C**)
	<105 μm	37.4±1.0(A**)	30.8±0.7(A**)	39.9±0.7(A**C**)
With the addition of ADF				
Fractions:	260-150 μm	37.3±0.8(A**)	30.3±0.6(A**)	39.2±0.9(A**B**)
	150-105 μm	38.2±1.0(B**)	30.1±0.3(A**B**)	38.2±0.9(A**B**)
	<105 μm	39.3±1.2	29.7±0.8(A**)	38.9±1.2(A**)

A - significant difference between the sample with fiber and the control sample

B - significant difference between the sample with DF and the sample with ADF

C - significant difference between samples with different fiber particle size

* - P = 0.05;
** - P = 0.01.

Table 2. Ratio of tyrosine by enzymes in investigated samples to that released in control samples (%).

Sample		Pepsin	Trypsin	Chymotrypsin
With the addition of DF				
Fractions:	260-150 μm	99.0	94.4	93.3
	150-105 μm	93.4	97.3	98.0
	<105 μm	95.7	91.4	88.5
With the addition of ADF				
Fractions:	260-150 μm	95.4	89.9	86.9
	150-105 μm	97.7	89.3	84.7
	<105 μm	100.5	88.1	86.3

It follows from the experiment that the inhibition of casein proteolysis depends on the kind of fiber. The amount of tyrosine released from casein by trypsin and chymotrypsin was lower in the case of DF addition than in the case of ADF addition. This statement relates to fraction 150-105 μm, and for chymotrypsin also fraction 260-150 μm.

The influence of fiber particle size on the enzymatic hydrolysis of casein was obvious for the DF addition with the use of pepsin. In the case of enzymatic reaction with the use of chymotrypsin, significant differences were observed between samples with the addition of each DF fraction; the greatest proteolysis inhibition was found for fraction <105 μm.

The ratio between the mean values of released tyrosine in the samples ranged from 84.7% to 100.5% (Table 2), while inhibition - from 10% to 15% at ADF addition to samples with trypsin and chymotrypsin and for fraction <105 μm with DF addition using trypsin. In the remaining samples, the inhibition of casein proteolysis ranged from 0% to 10%.

The varied influence of dietary fiber and acid detergent fiber results from their different chemical composition. Dietary fiber mainly consists of hemicellulose, cellulose and lignin, while acid detergent fiber contains no hemicellulose. Amarowicz and Fornal (1987) reported that buckwheat dietary fiber contained 22.8% of hemicellulose, 37.3% of cellulose and 39.9% of lignin.

The influence of fiber particle size on the course of protein proteolysis observed during the experiment is probably a result of the varied contact between the active centre of the enzyme and the substrate.

However, the problem requires further investigation, which is confirmed by the results of Ikeda et al. (Ikeda et al. 1986 and 1989). They suggested that the increase in the trypsin-inhibitory activity of various dietary fibers was greater when the fiber had been added to the substrate prior to the addition of the enzyme. In this context there appears a question about the nature of the complexes forming and their influence on enzymatic activity. A complement to this problem might be Acton's suggestion that dietary fiber may reduce protein digestibility through ionic interaction, matrix restriction, and modification of filtration characteristics by the fiber (Acton et al. 1982).

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An appraisal of the hormonal basis of grain growth in buckwheat (*Fagopyrum esculentum* Moench)

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Key words: endogenous hormones, sink size, yield

Abstract

Studies conducted on the growth and development behaviour of grains of Fagopyrum revealed the existence of a disparity with regard to size and their capacity to accumulate dry matter. By and large, there were two types of grains (bolder and smaller) which were distinguishable from each other by their weight and volume. The relative status of their endogenous hormones indicated that the variation in grain weight was traceable to the distribution pattern of different endogenous growth regulators. Three different auxins (IAA and two unknown) and two cytokinins (zeatin and 2iP) were detectable in all the grains. However, the bolder grains possessed significantly higher levels of these substances. The exogenous application of these growth promoters led to vigorous enhancement in dry matter gathering capacity of those grains which were smaller in size. The level of gibberellin, on the other hand, (GA_3 and GA_7), was not associated with grain weight. However, it altered another important facet determining the sink size i.e., fertility (grain number/ear). It was concluded that the relative contribution of different endogenous hormones was the major factor determining the differences in grain weight and/or sink efficiency in Fagopyrum.

Vloga hormonov za rast zrn pri ajdi

Raziskava razvoja in rasti zrn ajde je pokazala razlike glede zmožnosti kopičenja sušine v zrnih in rasti zrn. Večja zrna se od manjših razlikujejo glede na maso in volumen. Raziskava relativnih odnosov endogenih hormonov je pokazala, da lahko razlike v masi zrn povežemo z razlikami v endogenih regulatorjih rasti. Kvalitativno so bili ugotovljeni trije različni avksini (IAA in dva neznana) in dva citokinina (zeatin in 2iP) v vseh zrnih, toda njihove relativne količine so različne; večja zrna so imela več teh snovi. Tretiranje manjših semen z omenjenimi rastnimi hormoni je povečalo njihovo sposobnost akumuliranja sušine. Nasprotno pa nivoja giberelinov (GA_3 in GA_7) niso mogli povezati z maso semen. Videti pa je, da so povezani s številom zrn na socvetje. Ugotovljeno je, da je relativna razporeditev različnih endogenih hormonov pomemben dejavnik, ki določa razlike v teži zrn oziroma v učinkovitosti ponorov asimilatov pri ajdi.

Introduction

Buckwheat (*Fagopyrum esculentum*) has immense economic potential due to its protein rich grains, plant hardiness, short growth period and the use of the foliage as a green vegetable. An examination of the various physiological attributes governing its grain yield, especially with regard to endogenous hormones vis-a-vis metabolic profile, is likely to shed new light on its yield ability and hence may prove beneficial in realising a higher harvest index. A review of research into the physiological basis of grain growth reveals that Percival (1921) was perhaps the first to report that grains within the same ear of cereals or related crops might be of different sizes and he opined that this difference might be due to some physiological attributes operating within them. Since then,

a number of investigations have been carried out to evaluate the relative importance of diverse physiological processes and their contribution to grain growth or yield. Lupton (1966) working on wheat and Hulquist and Eastin (1970) working on sorghum, concluded that an efficient translocation system invariably contributed towards higher yield. On the other hand, Wardlaw (1968) and Bremner (1972), emphasised the ability of individual grains to grow and precipitate carbohydrates rather than the translocation, as the major determinant of seed growth. Asana (1974) argued that since the growth rates of grains of wheat within the same ear and variety differed during the early stages of the ontogeny of grain, when assimilates were not limiting, some inherent factor(s) must control the growth of these grains. Working on the hormonal control of sink efficiency in wheat, Bhardwaj and Dua (1974, 1975), Dua and Bhardwaj (1979a, 1979b) and Dua et al. (1982), showed that variation among varieties with regard to 1000 grain weight was traceable to the endogenous auxin and cytokinin production of the variety vis-a-vis that of the ear. It was unequivocally shown (Dua and Seghal, 1981), that heavier grains possessed a significantly higher combination of auxin and cytokinin than peripheral smaller grains. This postulation was further confirmed by culturing heavier and smaller grain in a standardised nutrient media, where it was shown beyond doubt (Dua and Kalsi, 1989), that the size of the grain was determined mainly by the endogenous status of hormones and it was this factor which governed the yielding ability in a single grain or ear and in totality determined the variability amongst the different grains of the same ear. A situation analogous to that of Triticum seems to exist in minor cereals like buckwheat, where variation amongst genotypes or different grains is also prevalent. Tahir and Farooq (1983, 1985) reported that two species of Fagopyrum viz. *F. esculentum* and *F. sagittatum* yielded poorly due to a plethora of diverse factors. The yield was correlated to flower abortion and/or the presence of mature and immature grains simultaneously at the time of harvest along with precocious germination of grains. Other workers, such as Adachi et al. (1983), have also attempted to interrelate its yielding ability with multifarious events, and various constraints determinant of economic yield have been isolated. Working on these lines, Sugawara (1960) opined that translocation of carbohydrates to the developing grains might be a limiting factor during grain setting, while Morton (1966) suggested that low grain yield in Fagopyrum could not be attributed to the lack of pollination, non-viable pollen, self-incompatibility mechanism or any other limiting cause and that some unknown factor(s) responsible for poor yield might be operating in these grains. Work done in the last two decades in production physiology has revealed that the yield capacity in crops is not only influenced by the availability of photosynthates but mainly by their sink efficiency i.e., the inherent capacity of grains to grow, and each grain might be endowed with its own production potential (Dua and Kalsi, 1990). Since a situation of differential growth and dry matter precipitation potential linked with poor yield has been prevalent in the grains of Fagopyrum, it was considered worthwhile to look into the relative components of sink size along with a concomitant probe into the relative levels of endogenous hormones in these unequally growing grains. It is further proposed to examine the effect of exogenous application of growth regulating substances on their dry matter precipitation capacity (harvest index).

Materials and methods

Plants of *Fagopyrum esculentum* were raised in pots (40x30x30 cm) containing 30 kg of soil mixed with FYM, (Farm Yard Manure) at Saproon Valley (H.P.) in the lower Himalayas. Twenty seeds per pot were sown and sixteen days later, seedlings were thinned to eight, with the tagging of mother shoots. The plants were grown with an optimum

supply of water and nutrients and were given a dose of Hoagland Solution at 10 day intervals. The date of anthesis was noted and the growth rate of different grains was recorded from eight days after anthesis until maturity. The grain volume was measured by the water displacement method as reported by Dua et al. (1983).

For the extraction of growth regulating substances, samples from individual mother shoots were pooled and divided into different lots. Grains from the first fertile floret characterised by a higher precipitating grain weight and volume were grouped as B (Bolder) grain and similarly, grain possessing significantly lower grain weight and volume, was apportioned as S (Smaller) grain. Grains of the same age were used for the estimation of different endogenous hormones. The subsequent analysis, involving the extraction and estimation of plant growth regulating substances, was carried out at the Department of Botany, Panjab University, Chandigarh. The samples were extracted with 80 per cent ethanol at 0°C in a refrigerator over a period of 24 hours. The extract was evaporated under suction to remove the ethanol and the aqueous phase was utilized for the extraction of various growth regulating substances. The auxins were extracted by the method of Nitsch (1956) and gibberellins by the procedure given by Murakami (1966). The purified extracts were chromatographed on Whatman Filter paper No. I, using iso-propanol : ammonia : water (10:1: 1 v/v/v) as a solvent for the developing chromatographs. The different gibberellins, GA3, GA5, GA7, GA9, and auxin (IAA) were run separately and the different Rf zones were bioassayed to detect their activity. The qualitative and quantitative estimation of cytokinins was carried out by a comprehensive scheme standardised previously in this laboratory (Dua and Jandiak, 1979). Standard series comprising zeatin riboside, zeatin ribotide, zeatin and 2iP were also run simultaneously on separate papers under identical conditions. All Rf zones, eluted with 0.3N CO_3COOH were tested for cytokinin activity by the *Xanthium strumarium* leaf disc senescence technique of Osborne and McCalla (1961). Estimations of auxin activity were carried out by use of the coleoptile straight growth test as described by Mer et al. (1962) with 'Kent' oat (*Avena sativa* L.) coleoptiles, gibberellins by the modified technique of Ogawa (1963) using rice (*Oryza sativa* L.) cultivar 'Tainen-3' seedlings. The data were analysed statistically according to the analysis of variance method.

Results

1. Studies on grain growth

A close scrutiny of the data available from growth studies on the grains of Fagopyrum (Fig. 1) revealed the existence of two types of grains. The two types differed significantly in their weight and volume at all stages of grain development. At 8 days after anthesis, grains categorised as bolder were approximately 51.7 per cent heavier than grains growing nearby and classified as smaller grains. Similarly, these bolder grains were 57.8, 64.1 and 68.5 percent heavier than their counterparts (smaller grains) at 15 and 22 days after anthesis and at maturity respectively. Volume estimates revealed that bolder grains were 13, 16, 19 and 20 percent more voluminous than smaller grains at 8, 15 and 22 days after anthesis and at maturity respectively. In addition, the heavier grains grew consistently faster than the smaller grains during all stages of grain development.

2. Endogenous distribution of growth regulating substances in the different grains:

2A. Auxins

The pattern of auxins (per unit fresh weight) in different grains showed that three auxins (Rfs 0.1-0.2, auxin-I; 0.6-0.7, auxin-II; and 0.9-1.0, auxin-III) were present in both types of grain and their relative distribution, along with total auxin, is given in Fig. 2. Auxin-II, which matched synthetic IAA,

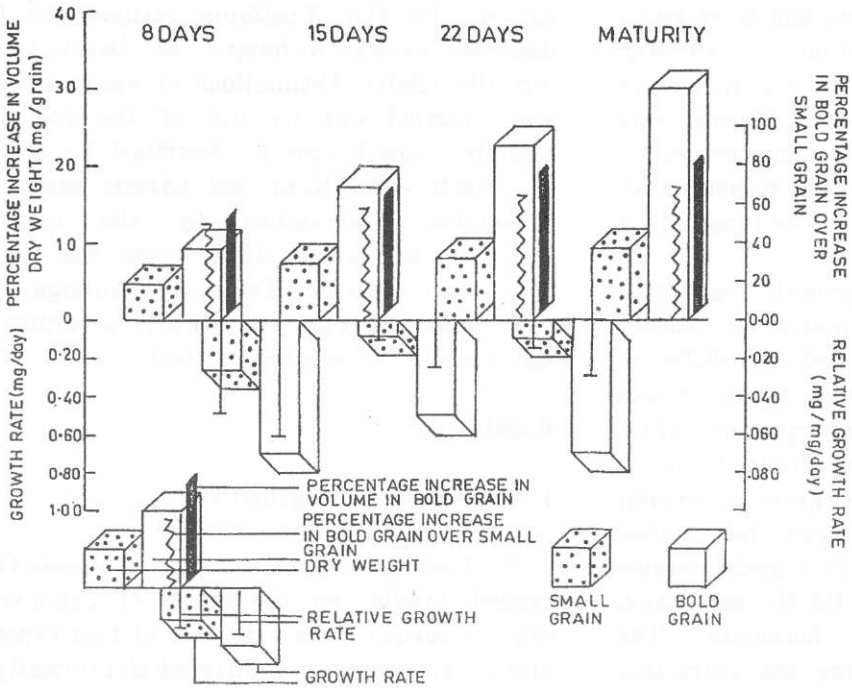

Fig. 1: Dry weight (mg/grain), growth rate (mg/day), relative growth rate (mg/mg/day) of bold and small grains as well as percent increase in dry weight and volume in bold grain over small grain of *Fagopyrum esculentum* at different intervals of time (growth stages) after anthesis. (Mean of five replications.)

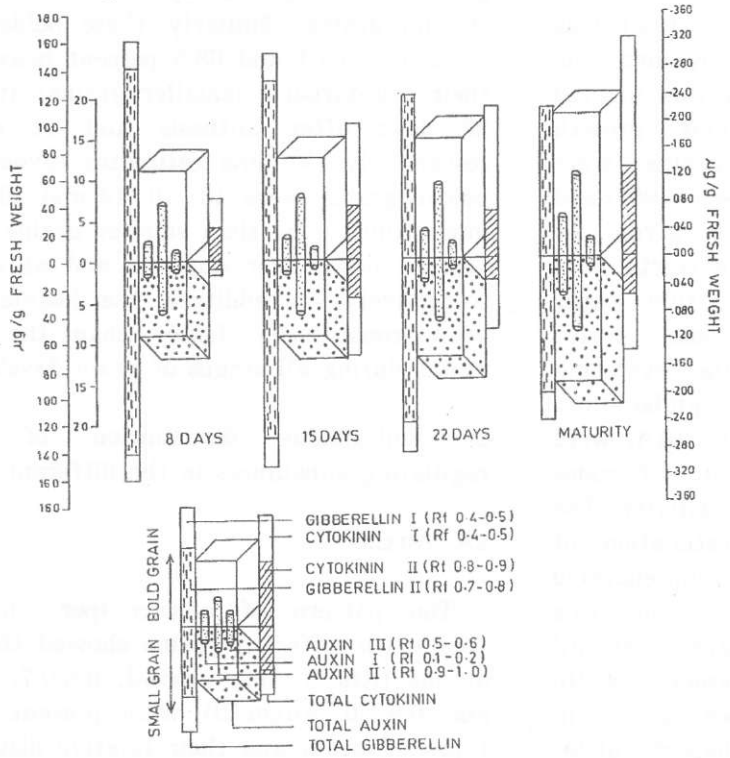

Fig. 2: Auxin, gibberellin and cytokinin activity in bold and small grains of *Fagopyrum esculentum* as revealed by different bioassays. (µg/g fresh weight. Average of five replications.)

constituted the bulk of total auxins. All auxins, irrespective of their chemical structure or the grains in which they were present, increased with the age of the grains. Further, the bolder grains had 17.3, 24.4, 17.2 and 13.7 percent higher total auxins as compared to the smaller grains at 8, 15 and 22 days after anthesis and at maturity respectively. In terms of the distribution of individual auxins in the two types of grains, differences in the levels of indole-acetic-acid were more pronounced as compared to the other two auxins, and were significantly higher in the bolder grains.

2B. Gibberellins

Two gibberellins (Rfs 0.4-0.5 and 0.8-0.9) were found to be present during the earlier stages of development of grains of Fagopyrum. The former matched synthetic GA3 while the latter corresponded to GA7. However, GA7 was not detectable during the advanced stages of ontogeny and in general, GA3 constituted the bulk of total gibberellins (92 percent of the total during the earlier stages and 89 percent in the advanced stages). The total gibberellins decreased as the grains progressed towards maturity and, surprisingly, there was no significant difference in their quantity in the two types of grain. It appeared that this endogenous hormone plays no major role in determining individual grain weight but had a definite role in determining sink efficiency through another parameter (grain number), which is discussed elsewhere in this paper.

2C. Cytokinins

Data on total as well as individual cytokinins revealed the existence of two cytokinins, at 0.4-0.5 and 0.8-0.9 Rfs, matching zeatin ribotide and 2iP respectively. In both types of grain, the total cytokinin increased as the grain progressed towards maturity. As apparent from Fig. 2, the bolder grains possessed 78.6, 28.2, 49.1 and 57 percent higher cytokinin as compared to the smaller grains, at 8, 15 and 22 days after anthesis and at maturity. Zeatin ribotide comprised 50, 40, 29, 40 percent of the total cytokinins present in both types of grain, at 8, 15 and 22 days after anthesis and at maturity respectively, while the rest was contributed by 2iP.

3. Effect of exogenous application of growth regulating substances on grain growth/grain number:

Exogenous application of various growth regulating substances offered some interesting revelations with regard to grain weight and/or grain number per ear. As apparent from Fig. 3, the application of auxin or cytokinin significantly improved the 1000 grain weight capacity and the smaller grains responded more vigorously to the exogenous supplementation of auxin or cytokinin than the bolder grains. The application of gibberellin did not affect the single grain weight component significantly. Nevertheless, gibberellin application improved another desirable facet of sink efficiency i.e., the number of grains/ear. The improvement in fertility per ear was to the order of 40-45 percent and wherever gibberellin was present in combination with auxin or cytokinin, this parameter (grain fertility) improved significantly.

Discussion

The potential of a grain to grow and accumulate photosynthetic assimilates has been recognised as an important parameter determining grain yield in many crops. Percival (1921) suggested that the difference in grain yield was attributable to either (i) a longer period for starch deposition in some grains; (ii) hormonal interaction amongst grains or (iii) competition for assimilates among grains. Kiesselback (1948) added another component to this list i.e., higher translocation rate. In addition to these parameters, a number of reports have also favoured other miscellaneous factors such as

Fig.3

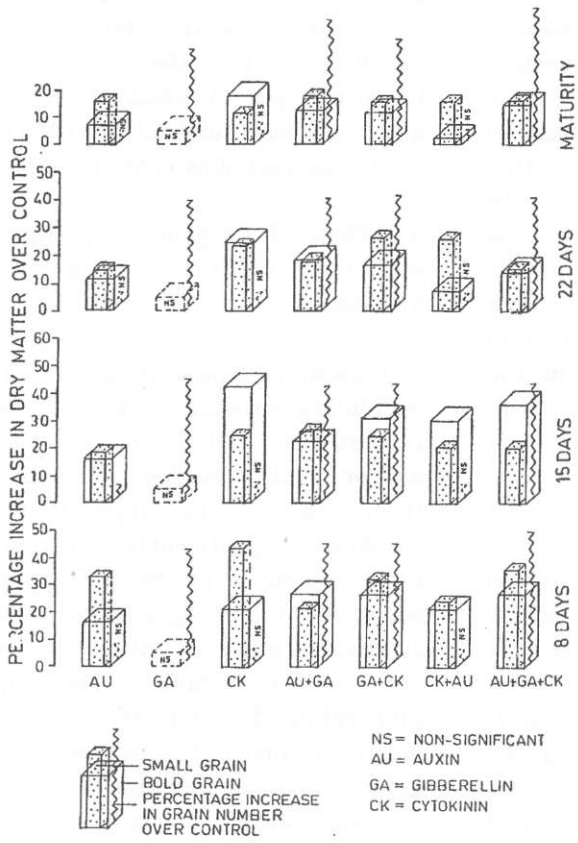

Fig. 3: Percentage increase in dry matter over control of individual grains at different positions within the same ear of *Fagopyrum esculentum* under the influence of growth regulating substances at different intervals of time. The figure also depicts the percentage increase in grain number over control under the influence of different growth regulators. (Average of five replications. Non-significant values are either dotted or/ and specified.)

photosynthesis (Birecka and Wlodkoswaska, 1966), leaf area duration (Wellback et al., 1966), or leaf area (Watson, 1952), which singly or jointly imparted a higher yielding ability to a cultivar. Research conducted in the last three decades (Asana and William, 1965; Asana et al., 1969; Bingham, 1969 and Rawson and Ruwali, 1972) has shown that the primary determinant of yield in wheat is sink efficiency (build up by grain size and number). Subsequent work done on these lines (Dua and Bhardwaj 1979a, 1979b; Dua, 1980) has shown that differences in varietal yield were traceable to differences in the endogenous growth substances viz., auxins, gibberellins and cytokinins. A correlation between grain size and auxins and cytokinins on the one hand, and gibberellin and grain number on the other, has been established. A further probe into the physiology of these more active and efficient sinks (heavier and faster growing grains) revealed the existence of a different metabolic profile in operation in them and ultimately these metabolic events have been linked with the relative distribution of hormones in these grains (Dua et al., 1983). In another study carried out under in vitro conditions (Dua and Kalsi, 1989), it was unequivocally shown that the smaller grains remained small even under optimum conditions of nutrient supply, thus ruling out the possibility of a nutritional

barrier in these grains. Based on these and other reports, the heavier and smaller grains were considered as independent biological entities. Before the implications of the present results on Fagopyrum are discussed and conclusions drawn, it is worth recapitulating the salient features of the results given in the preceding section. The results show that the minor cereal *Fagopyrum esculentum* is also characterised by a variable size of grain and differential yield. It was clear from an examination of endogenous levels of hormones, that the smaller grain possessed relatively low levels of auxin and cytokinin and any exogenous application of the same significantly augments their dry matter precipitation ability. In addition to this, the application of auxin or cytokinin also enhanced the yield ability of the bolder grains but this augmentation, though significant, was less than that available in the smaller grains. From the foregoing, it appears that a threshold level of auxin and cytokinin in grains is required to sustain an optimum dry matter accumulation capacity and these levels seemed to be sub-optimal in the smaller as compared to the bolder grains. Gibberellin on the other hand, had a significant role in deciding grain number and this showed marked improvement when gibberellin was applied exogenously. The individual observations of Adachi et al. (1983) and Morton (1966) that poor grain number in Fagopyrum was not due to the lack of pollination and that of Kusiorska and Koszykowska (1981), who concluded that failure in seed setting in Fagopyrum was due to some unseen events following pollination, are relevant in the present context. It seems probable that at the commencement of grain setting, a hyper-endogenous level of gibberellin might be responsible for giving the initial momentum to grain setting whereas subsequent development is regulated by auxin/cytokinin combinations. Data on exogenous application of gibberellin enhancing the grain number significantly seems to substantiate this conjecture. Reports showing exogenous application of growth regulating substances to enhance the grain yield of different crops have emanated from a few laboratories (Dua et al., 1982; Dua and Bhardwaj, 1972a, 1979b; Dua, 1980 and Sweet and Wareing, 1966). However, the present study involving the assessment of sink efficiency in relation to its components (grain number x grain size) correlative to its endogenous hormones and their adjustment exogenously is probably the first of its kind in Fagopyrum. The enhanced endogenous levels of auxin and cytokinin available internally to bolder grains or through exogenous means to smaller grains may enhance the transportation rate (Mullins, 1970) and storage capacity of the developing grain as advocated by Humpries (1963), Sweet and Wareing (1966) and Dua et al. (1989), thus paving the way for the transformation of a poorly developing sink to a relatively better sink.

To conclude, it is proposed that the differential yield ability amongst different grains of Fagopyrum seems to be related to the levels of auxins vis-a-vis cytokinins, while grain number appeared to be determined by the extent of production or availability of gibberellins.

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Quantitative amino acid analysis of buckwheat by reverse phase liquid chromatography

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Key words: HPLC, o-phthalaldehyde derivatives

Abstract

The amino acid composition was determined in two buckwheat cultivars, 'siva' and 'darja'. Amino acids were separated as o-phthalaldehyde derivatives by high performance liquid chromatography (HPLC).

Kvantitativna določitev amino kislin ajde s tekočinsko kromatografijo visoke ločljivosti

Določili smo aminokislinsko sestavo dveh sort ajde 'siva' in 'darja'. Med obema sortama ni bilo bistvenih razlik v aminokislinski sestavi. Analizni postopek je temeljil na ločitvi o-ftalaldehidnih derivatov amino kislin s HPLC na reverzno fazni koloni. Detekcija derivatov je bila fluorescenčna. Metoda je primerna za določanje primarnih amino kislin.

Introduction

Buckwheat is used as a cereal in the human diet. It has exceptional protein quality among plants, the high biological value - over 90% - being due to the favorable amino acid composition of buckwheat proteins (Javornik et al., 1981; Eggum et al., 1984). The amino acid content of buckwheat has been determined as a whole and by solubility protein fractions (Pomeranz and Robbins, 1972; Javornik and Kreft, 1984). Lyman et al. (1956) used a microbiological assay method for determination of amino acids in buckwheat. Tkackuk and Irvine (1969) used classical ion-exchange chromatography, which is a long procedure. Pomeranz and Robbins (1972) performed an assay with an automatic amino acid analyser. HPLC is a very appropriate and useful method for determination of amino acids. The separation of free amino acids is performed on an NH_2^- column (Schuster, 1980), or on reverse phase columns (Iskandarani et al., 1984; Walker et al., 1987). The separation of derivative amino acids is mostly used in HPLC. The derivatives are then separated on reverse phase columns. Precolumn derivatisation of amino acids with o-phthalaldehyde/2-mercaptoethanol is a rapid and sensitive method of amino acid analysis (Sista, 1986). The reaction conditions are relatively mild, but no derivative is formed with secondary amines (proline). The derivative of amino acid cystin and/or cystein has a very low molar extinction.

Material and methods

Material:

Two buckwheat cultivars, 'siva' and 'darja', were used for the analysis. The cultivar 'siva' was grown in Kleče (1988) and 'darja' was grown in Žabnica (1988).

Methods:

The HPLC method was used for analysis. It is based on the separation of o-phthalaldehyde derivatives of amino acids on a reverse phase column.

Standard solutions:

The amino acids (Serva) were dissolved to give 1.0mM in 0.1M HCl. Mixtures were prepared from these solutions.

Hydrolysis:

The samples were defatted with hexane and hydrolysed with 6M HCl for 48 hours at 110°C (Bidlingmeyer, 1987). Acid hydrolysates were neutralised with 5M NaOH.

Derivatisation procedure (Sista, 1986):

Neutralised aliquots were exposed to the derivatisation conditions. Samples (20 µl) were mixed with 10 µl of the o-phthalaldehyde/2-mercaptoethanol reagent. After 1 min of reaction at room temperature, 200 µl of solvent A was added and the mixture was injected into the HPLC system. All amino acids except proline, which is a secondary amine, react under these conditions. Cystine has a very low molar extinction.

Chromatographic conditions:

The pumping system consisted of two LDC/Milton Roy Constametric III HPLC pumps with an MP 3000 System for gradient control. The Rheodyne injector had a 20 µl sample loop. The column, (250x4.6) mm, was packed with Spherisorb S 5 ODS2. Column effluents were monitored with a Shimadzu RF-530 fluorescence detector. The excitation wavelength was set to 330 nm. The emission was measured at 425 nm.

Mobile phases:

Buffers were prepared from p.a. grade salts and double distilled water. The pH of the buffer (0.05 M sodium citrate, 0.005 M disodium hydrogen phosphate) was adjusted to 6.5 ± 0.03. To 96% of this buffer, were added 2% tetrahydrofurane and 2% acetonitrile. Buffer B consisted of 63% methanol (LiChrosolv, Merck), 2% acetonitrile (LiChrosolv, Merck), and water (double distilled). All mobile phases were degassed daily with a vacuum pump. The gradient used was plotted on the chromatogram.

Results and discussion

Fig. 2 shows the chromatogram obtained for the mixture of standard amino acids. The separation of the critical pairs Thr and Arg, Tyr and Ala was achieved with the addition of acetonitrile to solvent B (Pečavar, 1989). Table 1 shows that the content of nitrogen, ash and moisture was similar in both samples.

Table 1: Characterisation of sample.

	'darja'	'siva'
moisture	9.11%	10.53%
ash	2.67%	2.99%
nitrogen	1.81%	1.78%

Chromatograms obtained for the samples of 'siva' and 'darja' are shown in Fig. 3a and b. There were no significant differences in the amino acid composition of the two cultivars (Table 2).

Table 2 and Figure 1 show that the amino acid composition of the two samples is very similar. The amino acids Cys and Pro cannot be determined in a single run (Cooper et al., 1984).

Conclusions:

This method is very suitable for the rapid analysis of essential amino acids.

The separation of the critical pairs can be improved by the addition of acetonitrile to solvent B.

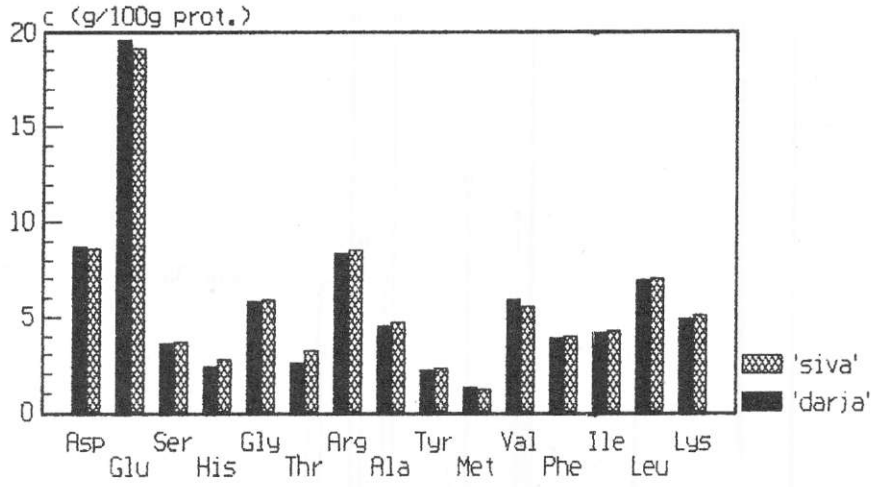

Figure 1: Amino acids in buckwheat 'siva' and 'darja'

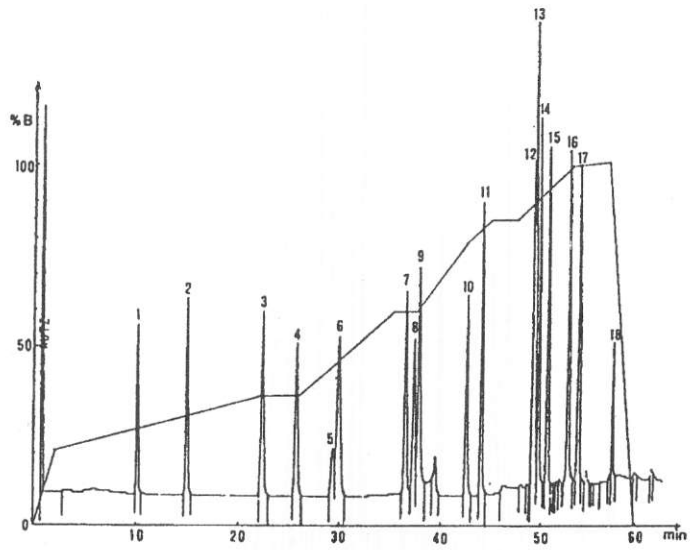

Figure 2: Chromatogram of standard mixture of amino acids. The separation of OPA/MCE derivatives of amino acids was performed on a Spherisorb S 5 ODS2, (250x4.6) mm column. Solvent A: 0.05 M sodium citrate, 0.005 M disodium hydrogen phosphate, pH 6.5, 2% of tetrahydrofuran, 2% of acetonitrile. Solvent B: methanol, acetonitrile, water (63:2:35). 1-Asp, 2-Glu, 3-Asn, 4-Ser, 5-His, 6-Gln, 7-Gly, 8-Thr, 9-Arg, 10-Ala, 11-Tyr, 12-Trp, 13-Met, 14-Val, 15-Phe, 16-Ile, 17-Leu, 18-Lys.

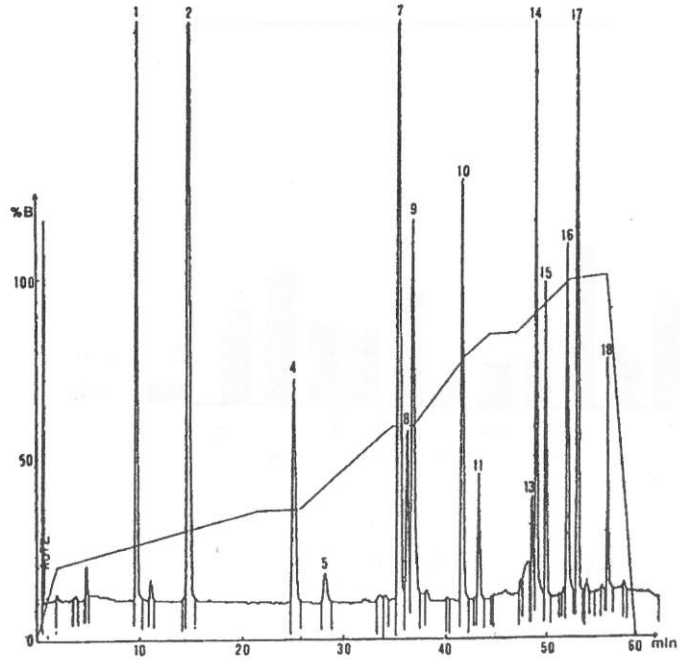

Fig. 3a

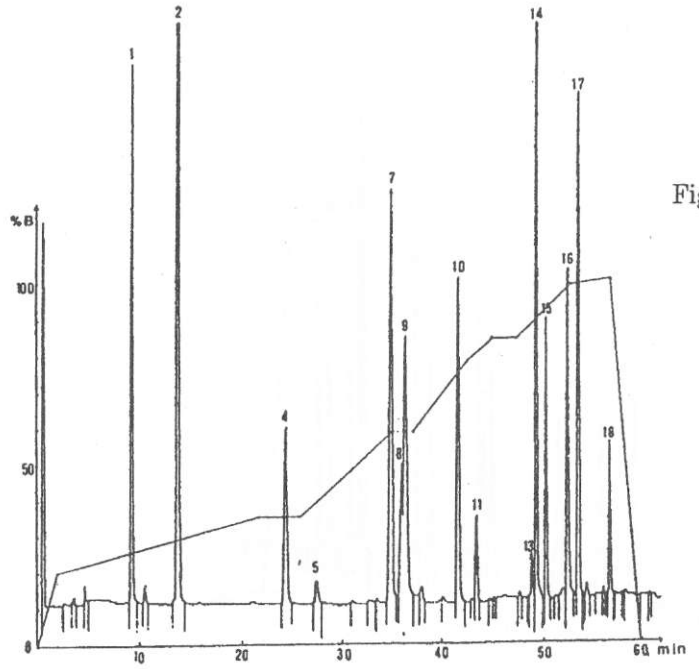

Fig. 3b

Figure 3a and 3b: Chromatogram of the hydrolysate of buckwheat a) 'darja' and b)'siva'. Separation of OPA/MCE derivatives of amino acids was performed on a Spherisorb S 5 ODS2, (250x4.6) mm column. Solvent A: 0.05 M sodium citrate, 0.005 M disodium hydrogen phosphate, pH 6.5, 2% of tetrahydrofuran, 2% of acetonitrile. Solvent B: methanol, acetonitrile, water (63:2:35). 1-Asp, 2-Glu, 4-Ser, 5-His, 7-Gly, 8-Thr, 9-Arg, 10-Ala, 11-Tyr, 13-Met, 14-Val, 15-Phe, 16-Ile, 17-Leu, 18-Lys.

Table 2: Amino acid composition of buckwheat samples (gAA/100g proteins).

Amino acid	'darja'	'siva'
Aspartic acid	8.7 ± 0.4	8.6 ± 0.4
Glutamic acid	19.6 ± 1.1	19.1 ± 1.1
Serine	3.6 ± 0.2	3.7 ± 0.2
Histidine	2.4 ± 0.2	2.7 ± 0.2
Glycine	5.8 ± 0.2	5.9 ± 0.2
Threonine	2.6 ± 0.2	3.2 ± 0.2
Arginine	8.3 ± 0.3	8.5 ± 0.3
Alanine	4.5 ± 0.3	4.7 ± 0.3
Tyrosine	2.2 ± 0.2	2.3 ± 0.2
Methionine	1.3 ± 0.1	1.2 ± 0.1
Valine	5.9 ± 0.4	5.5 ± 0.4
Phenylalanine	3.9 ± 0.3	4.0 ± 0.3
Isoleucine	4.1 ± 0.3	4.2 ± 0.3
Leucine	6.9 ± 0.5	7.0 ± 0.4
Lysine	4.9 ± 0.5	5.1 ± 0.6

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Buckwheat in Nepal[1]

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Key words: *Fagopyrum cymosum*, *Fagopyrum esculentum*, *Fagopyrum tataricum*, cultivars, cultivation, utilization

Ajda v Nepalu

V preglednem članku avtor poroča o načinu pridelovanja ajde in o njeni uporabi v Nepalu. Prikazani so načini vrstenja poljščin in kako je v vrstenje vključena ajda. Preizkusili so različne vzorce ajde, rezultati poskusnega pridelovanja so prikazani v razpredelnicah.

Introduction

Buckwheat (*Fagopyrum* spp.) is one of the staple food crops of the mountain people of Nepal, where it is the sixth most important crop after maize, rice, wheat, finger millet and barley, and the main crop in certain high mountain pockets where the Sherpa (people of Tibetan origin) live. To date, buckwheat has been an under-exploited, poor people's or neglected crop in Nepal. This pseudo-cereal (called KUANNA = Ku = bad and ANNA = cereal in Nepali) generally receives low inputs, little care and a low priority from the farmers themselves. However, it is an important crop since it is grown in moisture, nutrients and temperature stress conditions, in very remote and food deficient mountain areas of the country. It has a very short growth period, wide adaptability and is thus grown as a catch crop in different agro-climatic areas and soil types in Nepal.

Cultivation

Buckwheat is mostly cultivated in marginal upland areas with sloping infertile land on which other crops cannot be grown. However, in high mountain areas, farmers grow this crop on the better land and apply adequate compost.

Buckwheat planting starts in May at elevations above 2800 m and continues up to September at lower altitudes. In the southern plain area, this crop is planted from Mid-November to Mid-December. Depending on altitude, buckwheat takes from two to four months to mature in Nepal. In certain mountain areas, buckwheat is planted in December as a second crop and is harvested in March. Though it is generally considered an autumn crop, strictly speaking buckwheat is a summer crop in high mountain, an autumn crop in middle mountain and a winter crop in low mountain and plains areas.

In certain pockets pasture or bush forest is cleared, all plant materials gathered into piles and burned in close contact with the soil. Buckwheat is then planted by broadcasting after potato. Planting, interculture, harvesting etc. operations are carried out manually by the most primitive methods. It is cultivated without the application of fertilizers or plant protection measures.

Utilization

Nepalese farmers dehusk the tartary buckwheat with the aid of a locally made

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[1] Paper presented at the 4th International Symposium on Buckwheat, Orel, USSR, July 10-15, 1989.

wooden machine (dhiki). They believe that this process reduces its bitterness. The buckwheat grains are ground either by water mill or hand mill, and the flour is screened two to three times to remove the hull.

Sweet buckwheat is mainly used to prepare different types of breads; and tartary buckwheat is used for the preparation of porridge and breads. To improve the taste of tartary buckwheat, it is mixed with the flour of sweet buckwheat, barley, wheat or finger millet. In certain pockets of Nepal, tartary buckwheat is used to prepare a local drink.

The tender leaves of wild and common buckwheat are used as a green vegetable and the straw is used as animal feed and bedding material. Most of the buckwheat production is consumed within the country, though it was recently reported that about 296 metric tons of buckwheat was exported at high value in 1986/1987.

Species found in Nepal

Two cultivated species *Fagopyrum esculentum* Moench (sweet or common) and *Fagopyrum tataricum* Gaertn. (bitter or tartary) and one wild species *Fagopyrum cymosum* Meissn. are found in Nepal. Bitter buckwheat is grown exclusively in high mountain regions above 1500 m. The sweet type is very common, and is extensively cultivated in middle and low mountain and to some extent in high mountain and plains areas. The wild type is perennial and occurs in the altitudinal range of 1500m to 3000m mainly beside rivers and trekking routes as a "companion" crop of tartary buckwheat. Dr. Ujihara has reported that most Nepalese common buckwheat varieties are of the autumn type, indeterminant growth and sensitive to day-length. Bitter buckwheat is more cold tolerant, higher yielding and fills the grains better than the sweet types.

Buckwheat germplasms of Nepal have widely varied genetic traits in terms of both quality and quantity. Some experimental data are presented in Table 1. Sweet and tartary types differ in maturity, plant height, test weight, seed setting, number of branches and clusters and grain yield (Table 1).

Some statistics

The area and production of buckwheat have not been recorded regularly in Nepal. A National Sample Survey estimated about 17000 hectares in 1971/1972 and 11000 hectares in 1981/ 1982, which indicates a decreasing trend in area. However, in 1985, the Land Resource Mapping Project estimated 23100 metric tons production of buckwheat from 43000 hectares, with an average yield of 540 kg/ha. Buckwheat cultivation is mainly concentrated in mountain areas, but recently its cultivation has been increasing in the plains area (Tarai). Buckwheat growing areas are shown in Figure 1.

Mountain cropping patterns and buckwheat

Cropping patterns in the Nepalese mountains are very complex, mainly due to the huge altitudinal ranges, different agro-climatic conditions and land types. Being a short duration crop with high adaptation, buckwheat fits uniquely into various cropping patterns. Some of the mountain cropping patterns with buckwheat as one of the components are given below:

A) High mountain: 2000 m to 4000 m, cool temperate climate

1. Rainfed upland

Patterns	Duration
maize - potato - tartary buckwheat	2 years
barley or wheat - potato - tartary buckwheat	2 years
tartary buckwheat - fallow	1 year
potato - tartary buckwheat	2 years
barley or wheat - buckwheat	1 year

2. Irrigated lowland

naked barley - buckwheat	1 year
potato - buckwheat	1 year
barley or wheat - buckwheat	1 year

B) Middle mountain: 1000 m to 2000 m, warm temperate to subtropical climate

1. Rainfed upland

maize/finger millet - buckwheat	1 year
maize - buckwheat	1 year
maize - buckwheat - wheat or barley	1 year

2. Irrigated lowland

rice - buckwheat	1 year
rice - buckwheat - buckwheat	1 year

C) Low mountain and plain: 300 m to 1000 m, subtropical to tropical climate

1. Rainfed upland

maize - buckwheat	1 year
maize - buckwheat - buckwheat	1 year

2. Irrigated lowland

rice - buckwheat	1 year

Buckwheat is grown in association with many other crops too.

Research work on buckwheat

Being a secondary or minor crop, buckwheat research has not until recently been systematically carried out. Some research was initiated in the early seventies by evaluating cultivars from Canada and Japan as well as local germplasm. The IR-13 variety (Japan) has been identified. Buckwheat research has received national attention recently, and research has been supported by international organizations and/or agencies since 1986. Since 1987, buckwheat has been one of the mandate crops of the "National Hill Crops Improvement Program (NHCIP)", which is a fully-fledged National Commodity Program (Figures 3 and 4). Research is being coordinated from its headquarters at Kabre, with testing sites in different agro-ecological zones of Nepal (Table 3 and Figure 2).

The main thrust of buckwheat research is on varietal improvement to discover superior high yielding genotypes. NHCIP has been carrying out the following steps in its varietal improvement program:

- Collection and introduction of local and exotic germplasms.
- Evaluating these lines through an observation nursery, preliminary yield trials, and advanced varietal trials in multi-location tests.
- Conducting farm trials (FFT, PPVT) and demonstrations under field conditions.
- Variety release and seed conditions.

Germplasms from Canada, Japan, USSR, Poland, India, Yugoslavia, South Africa, China, Czechoslovakia, USA, Sweden and local sources were evaluated in Khumaltar, Kabre and Jumla. Based on selection criteria of better seed setting, more branching and clusters, bold and plump seeds, early to medium maturity and plant height, 23 lines at Kabre, 29 lines at Khumaltar and 11 lines at Jumla were selected. In addition to early maturity, a high percentage of seed setting and seed plumpness were observed in buckwheat lines from abroad. Most of the exotic materials showed lower plant height, better seed setting and earlier maturing than local germplasms, but the grain yield of local materials is generally higher than the exotic sources. Results are given in Table 2.

Recurrent and mass selection programs have already been started in Kabre.

Testing sites

The operational sites (Centre, sub-centre and testing sites), located mostly in mountainous zones (Table 3 and Figure 2), are intended to generate adaptable technologies which can then be disseminated within those regions to achieve as comprehensive a national coverage as possible. At present, simple trials are being conducted there. Kabre Agricultural Farm is the headquarters for coordination.

Organization

NHCIP is responsible to the Ministry of Agriculture through the National Agricultural Research and Services Centre (NARSC). The organization chart, a modified version of that

proposed by Mr. A.M. Pradhanang, is shown in Figure 3. The stations or farms which have been identified for hill crops research are shown.

NHCIP functions independently but works in close collaboration with the eight disciplinary divisions headquartered at Khumaltar and another twelve centres located in different parts of the country (Figure 3). The functional organization chart of NHCIP is given in Figure 4.

Technical constraints

Constraints to buckwheat research and production in Nepal include the following:

1. Most of the germplasms display poor seed setting and cold susceptibility.
2. Nepalese farmers are ignorant about the nutritive value and importance of buckwheat.
3. Since sweet buckwheat is a highly outcrossed species, it is very difficult to produce pure seed of many lines.
4. Most local cultivars are low grain yielding.
5. There is a lack of marketing facilities.
6. There are very few improved technologies and a shortage of the necessary inputs for buckwheat production.
7. Lodging, pests and diseases also limit buckwheat production in Nepal.
8. Tartary buckwheat is an important crop in Nepal, but research findings on this type are almost negligible.

Figure 1: Distribution map for buckwheat production

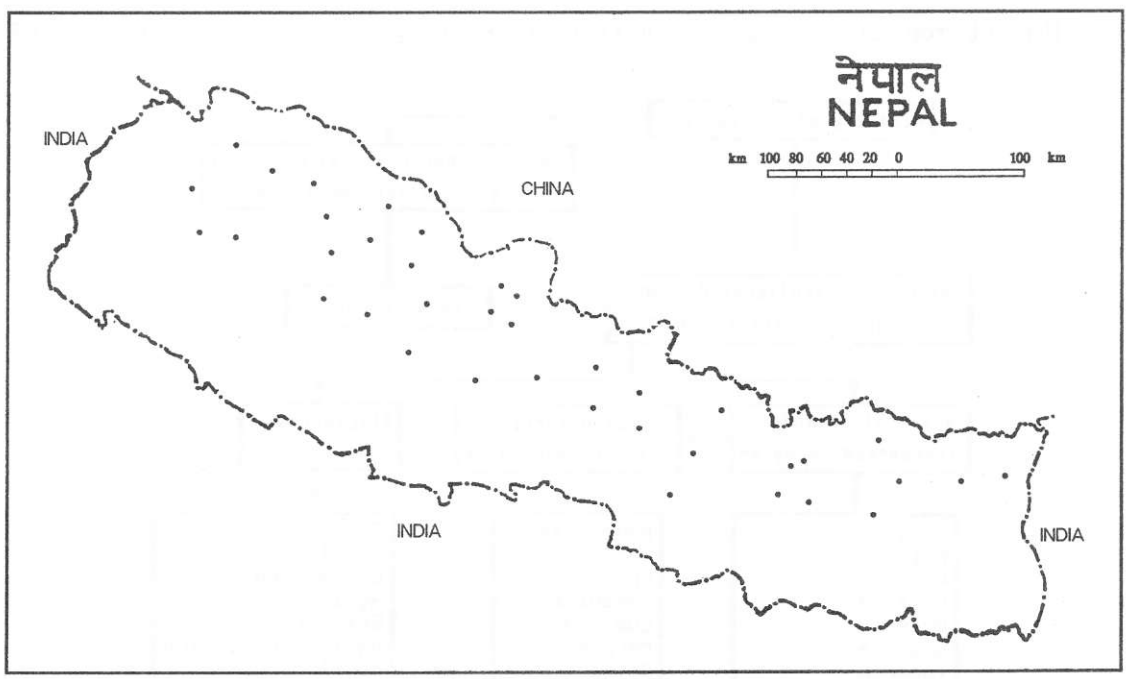

Figure 2: NHCIP research centres and testing sites

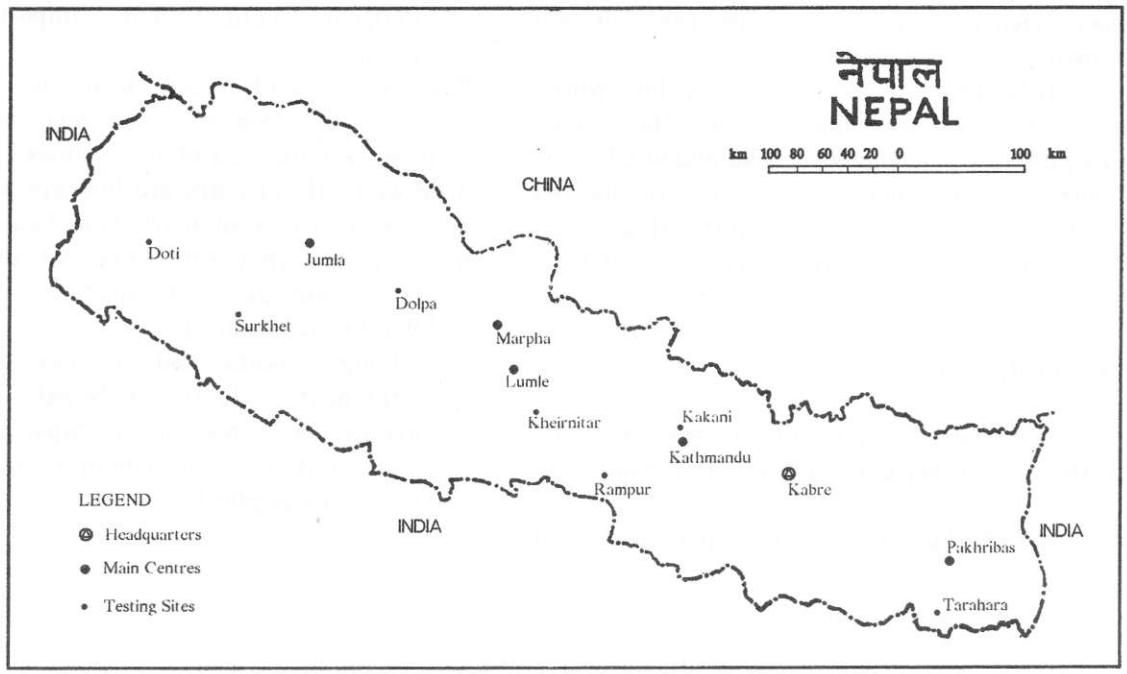

Figure 3: Present crop research organisation chart (Pradhanang, 1984, with some modifications)

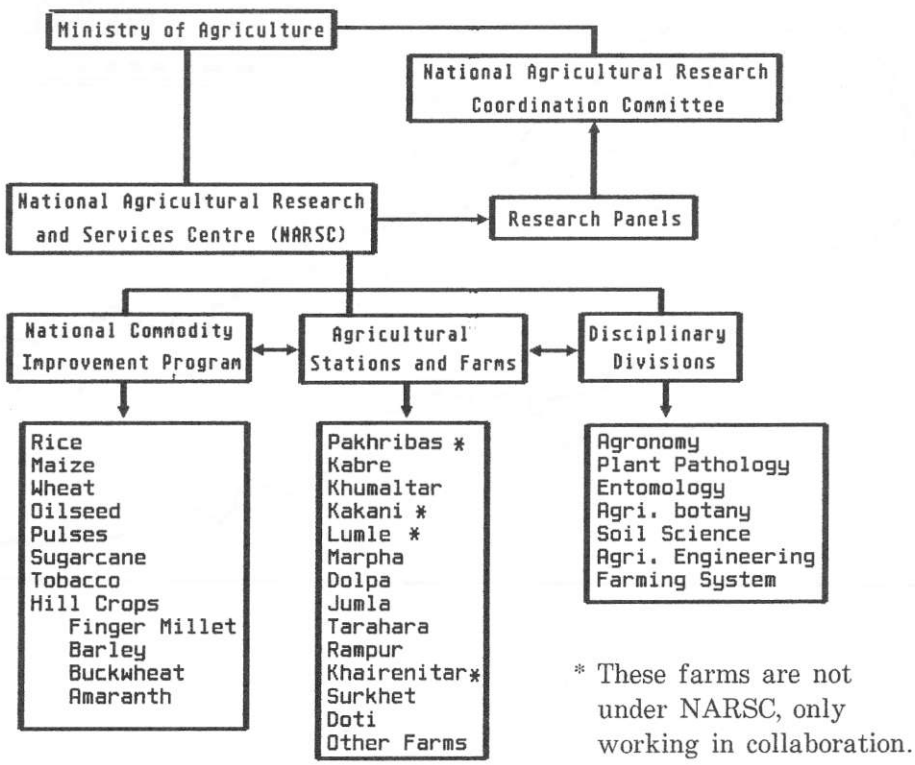

Figure 4: Hill crops program institutional framework and functions (Sherchan et al. 1986)

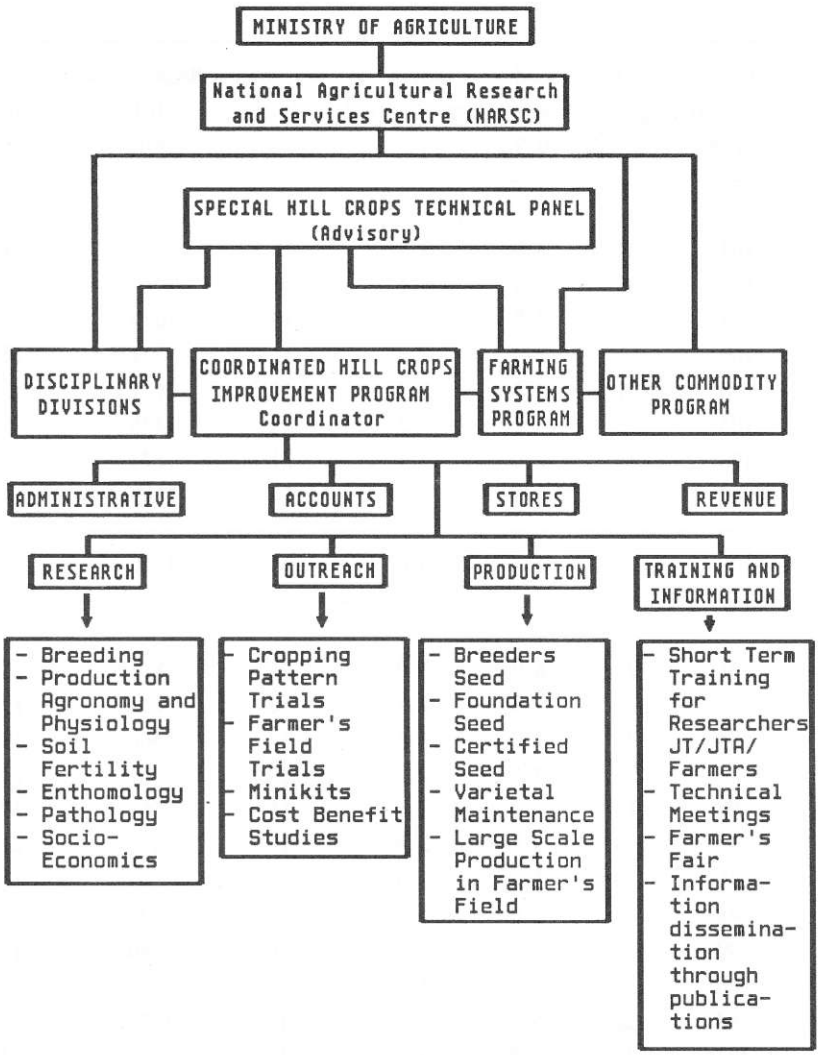

Table 1. Means of agronomical traits of sweet and tartary buckwheat (average of two locations, 1988/1989)

Cultivar	Flowering (Days)	Maturity (Days)	Plant height (cm)	1000 grain wt.(g)	Seed set (0-9)	No. of branch/plant	No. of cluster /plant	Mean grain yield (kg/ha)	Rank for yield
Sweet type									
22 Humla-S	25	70	104	30	3.0	3.5	10	1039	9
27 Humla-S	25	72	101	36	2.0	3.4	14	917	10
88 Humla-S	26	69	98	19	3.0	3.3	15	1079	8
126 Mustang-S	27	70	100	21	2.0	4.3	18	774	12
209 Mustang-S	25	66	93	31	3.0	3.7	17	1171	6
224 Tokyo-S	25	64	105	25	3.0	3.2	15	1475	5
246 IR-13-S	29	70	108	32	2.0	3.7	21	674	15
Local-S	25	66	89	27	2.0	3.5	10	1503	4
Mean	26	68	100	28	2.3	3.6	15	1070	-
Tartary type									
13 Mustang-T	35	77	91	20	6.0	3.0	21	818	11
26 Humla-T	36	78	83	22	5.0	2.7	16	645	17
38 Humla-T	38	75	84	26	3.0	1.5	17	566	18
46 Mugu-T	36	71	81	25	4.0	2.3	17	431	20
58 Mugu-T	36	72	88	19	4.0	3.4	15	561	19
62 Humla-T	38	73	90	19	5.0	2.6	19	428	21
68 Humla-T	38	71	75	20	5.0	1.9	15	649	16
113 Mustang-T	31	63	44	17	5.0	3.0	71	740	13
143 Jumla-T	36	74	90	20	3.0	2.7	16	680	14
177 Tanahu-T	38	94	80	31	8.0	1.1	8	1137	7
210 Khumal-T	34	84	88	22	6.0	2.5	16	1775	2
236 Mustang-T	34	83	102	13	8.0	1.3	13	1824	1
Local-T	24	65	88	11	5.0	4.3	16	1630	3
Mean	35	75	83	20	2.5	20	914	-	

Adopted from Initial Evaluation Trial, 1988/1989.

Table 2: Agronomical traits of some buckwheat germplasms

Cultivar	Country of origin	Flowering (Days)	Maturity (Days)	Plant height (cm)	1000 grain wt.(g)	No. of branch/plant	No. of cluster/plant	Grain yield (kg/ha)	Rank for yield
To-Matusoba	Japan	26	59	63	34	2.0	5	600	8
Kabre-S	Nepal	28	74	93	25	4.0	17	700	7
Shinshu-0-Soba	Japan	27	68	65	55	2.0	8	600	8
Shinano-Ichigo	Japan	27	61	71	33	3.0	5	1000	3
Siva	Yugoslavia	28	67	63	23	4.0	8	300	15
Humla-T	Nepal	38	84	77	17	6.0	25	1175	2
Darja	Yugoslavia	27	60	60	23	3.0	8	425	12
Shinano-Soba	Japan	26	59	65	28	2.0	5	775	5
Kabre-T	Nepal	28	84	75	15	5.0	28	1450	1
Mancan	Canada	36	60	63	29	3.0	5	388	13
GF 5239	Poland	26	61	65	20	3.0	8	725	6
Scorospelaya	USSR	25	61	65	20	3.0	11	600	8
Emka	Poland	28	81	65	36	3.0	9	350	14
IR-13 (Ckeck)	Japan	27	62	70	22	4.0	12	950	4
Humla-S	Nepal	28	75	83	26	4.0	19	550	11
Mean		28	68	69	27	3.4	12	706	

T - Tartary or Tite
S - Sweet or Mithe

F test	*
CV %	37
LSD (kg/ha)	580

Adapted from Preliminary Yield Trial, 1988/1989.

Table 3: Hill crops research stations in Nepal

Site	Region	Elevation (m)	Average rainfall (mm)	Temperature (°C) summer max.	summer min.	winter max.	winter min.
1. Pakhribas	Eastern	1760	2250	23.9	16.9	15.1	5.2
2. Kabre	Central	1740	1900	26.0	12.0	18.0	4.0
3. Khumaltar	Central	1360	1300	26.4	18.8	17.1	1.3
4. Kakani	Central	2030	2400	22.9	14.3	14.4	2.3
5. Lumle	Western	1670	5100	23.1	16.6	12.9	5.1
6. Marpha	Western	2610	450	20.7	12.4	10.9	-1.7
7. Dolpa	Mid-Western	2500	-	-	-	-	-
8. Jumla	Mid-Western	2387	970	23.9	13.8	14.0	-4.7

Other possible sites for buckwheat research:

1. Tarahara	Eastern	200	1700	32.8	23.2	27.4	12.5
2. Rampun	Central	228	2020	33.1	23.9	22.8	7.6
3. Khairenitar	Western	525	2301	32.1	21.4	25.5	12.0
4. Surkhet	Mid-Western	450	1500	30.1	22.3	19.3	5.4
5. Doti	Far-Western	620	1300	28.9	21.5	16.0	8.6

Source: Hill Crops Improvement Program in Nepal. Consultancy Report, 1986 and other sources.

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The problem of fertility in autotetraploid buckwheat. Report 1. Meiosis and fertility of experimental autotetraploids in *Fagopyrum esculentum* Moench.

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Key words: bivalents, 'Bolshevik', chromosome behavior, chromosomes, gametes, pollen mother cell, quadrivalents, univalents

Abstract

The nature of conjugation and chromosome behavior at the post-conjugation stages of meiosis in high and low fertility plants of 3 populations (A, B and C) of autotetraploid buckwheat were studied. At the start of the investigation, Population A plants had passed through a 17-stage selection for increased fertility. Population B plants were C_1-C_2 generation, derived from the diploid buckwheat variety 'Bolshevik', having passed through a 12-stage selection for fertility. The fertility of Population A and B plants was 81-87% of the fertility of the diploid variety. Population C was created on the basis of low fertility plants from Populations A and B, and their fertility was about 20% of that of the diploids.

It was demonstrated that in plants of Populations A and B, 95-97% of chromosomes and in plants of Population C, 90% of chromosomes formed bivalents. In population C, there was a greater number of quadrivalents and, particularly, univalents per one pollen mother cell in comparison to Populations A and B. A significant effect of quadrivalents and univalents on autotetraploid fertility was demonstrated.

The highest percentage of changed PCMs was in Population C plants and the lowest in Population B plants. Population A plants were intermediate.

On the basis of meiotic data, significant positive correlations were shown between the number of quadrivalents and univalents, and the principal types of disturbances at the post-conjugation stages of meiosis. It was concluded that the presence of unbalanced gametes in buckwheat autotetraploids is due to the nature of the chromosome conjugation. So selection is necessary, mainly for bivalent conjugation of chromosomes. Taking the example of Population B, we showed the important role of the initial diploid material involved in polyploidization.

Problemi fertilnosti avtotetraploidne ajde. 1. del: Mejoza in fertilnost eksperimentalnih avtotetraploidov ajde.

Raziskovano je bilo obnašanje kromosomov v mejozi pri rastlinah z visoko oziroma nizko fertilnostjo treh avtotetraploidne ajde. Populacija A je bila 17 generacij izbirana za visoko fertilnost rastlin. Populacija B so rastline C_1-C_2 generacije, dobljene iz diploidnega kultivarja 'Boljševik', ki so bile 17 generacij izbirane za fertilnost. Populacija C je zasnovana z nizko fertilnimi rastlinami populacij A in B, njihova fertilnost je bila samo okoli 20% fertilnosti diploidov. Proučevani so bili načini združevanja kromosomov v mejozi, razne citogenetske anomalije ter povezava s fertilnostjo rastlin. Rezultati, dobljeni s populacijo B nakazujejo, da je za uspeh dela s poliploidi zelo pomembna ustrezna izbira izhodiščnega diploidnega materiala.

Introduction

Experimental autotetraploids of *Fagopyrum esculentum* Moench were obtained in 1941 by V.V. Sakharov, S.L. Frolova and V.V. Mansurova from 9 varieties of diploid buckwheat in the USSR. However, the most promising for polyploidization was a diploid buckwheat variety - 'Bolshevik' (48-51). This form of buckwheat, as a variety of the autotetraploid 'Bolshevik-4', was later released for production.

In contrast to the initial diploid form, autotetraploids have greater plant vigor, larger and heavier kernels, less shattering and better resistance to lodging. The new form of buckwheat appeared to be more resistant to the effect of different abiotic and biotic factors, as well as to low temperatures, to the effects of ionizing radiation and some chemical mutagenes, and resistant to certain diseases. Autotetraploids have a greater content of some biologically important matter as compared to diploids, such as: nectar, rutin, etc. (9, 15, 49, 52).

However, the employment of these valuable characters of autotetraploid buckwheat in breeding practice is difficult due to its reduced fertility as compared with the initial diploid form, that is typical of most other autotetraploids, which are used for the sake of seed productivity. Firstly, most plants of autotetraploid buckwheat were superior to the diploid form considerably, the others were equal, and some of them were inferior to the initial form (48-49).

The problems of seed productivity in experimental autotetraploids have been discussed for a long time. Nevertheless, the problem of its renewal has not been solved as yet, nor have those mechanisms, which condition its reduction been cleared up (6, 8, 16, 22, 27-28, 38, 45, 53, 63-64, 67).

Problems of seed productivity in autopolyploids have been considered from cytogenetic (11-14, 18-19, 24, 30, 34, 38-39, 43, 56-57), embryologic (41-42, 66), morpho-physiological (7, 28, 55) and genetical (3, 17, 25-26, 37, 39-40, 43, 56, 60) points of view.

Plant fertility depends on the regular conjugation of homologous chromosomes in the early prophase of the first meiotic division and on the regular distribution of chromosomes at subsequent stages during macro- and microsporogenesis. Disturbances in these processes cause the formation of aneuploid egg-cells and pollen grains.

Autotetraploids differ from their corresponding diploids in that they have 4 identical haploid sets of chromosomes, which joint at their polarized ends and conjugate at full length at the beginning of Prophase I. The conjugation allows the approach of the homologous chromomere to the corresponding chromomere of the other. This explains why a corresponding number of quadrivalents should be formed in autotetraploids after chromosome conjugation. However, in most experimental autotetraploids, only some chromosomes form quadrivalents, while other chromosomes form respectively bivalents or trivalents and univalents, or they remain fully unassociated (13, 20, 32-33, 35).

The partial conjugation of homologous chromosomes observed in autotetraploids can theoretically lead to the following types of associations: quadrivalent, trivalent + univalent, 2 bivalents, bivalent + 2 univalents, and 4 univalents. The formation of multivalents may also be observed. On the basis of partial chromosome conjugation, Darlington suggests that the associations of homologues depend on the expression of synapsis and on chiasma frequency and position (11). This hypothesis was experimentally confirmed in further studies (21, 36, 47).

Chiasma formation depends on chromosome length and the duration of conjugation (10, 29, 54). The quadrivalent form is determined by the chiasma number and their localization as well as the degree of their terminalization (12-13). The co-orientation of centromeres (12, 58-59) affects the position of a quadrivalent in the equatorial plane of the spindle and the regularity of its divergence. In the process of a quadrivalent arising in the form of a ring, its position on the spindle will be such that pairs of centromeres face each pole (parallel

co-orientation). In this case, a regular divergence of chromosomes to poles (5, 65) can be observed. When a quadrivalent arises in the form of a chain (linear co-orientation), chromosomes will diverge random to the poles. Parallel and convergent co-orientations as a rule promote regular divergence of chromosomes. The process of chromosome divergence is broken in the presence of convergent co-orientation.

Trivalent formation from autotetraploids is correlated with a disturbance of conjugation and, as a rule, the mean trivalent number per cell is not large (19, 32). Trivalent behavior at the post-conjugation stage of meiosis depends on the position of the centromeres of the 3 chromosomes relative to each other and to the spindle. With linear and indifferent co-orientations, the two terminal chromosomes will go to the opposite poles and the middle one will usually remain on the equatorial plate. With convergent co-orientation, two chromosomes will move to one pole and the third to the other.

Univalents are randomly distributed to the poles at Anaphase I; at the division of a centromere in the first meiotic division, chromatids may go to the poles as daughter chromosomes and at the second division, be distributed to the poles; if the division of a centromere has not taken place during the first division, it does so during the second and chromatids can diverge to different poles, forming micronuclei; at the 1st and 2nd meiotic divisions, a breakage of a univalent through a centromere can be observed, which will result in the formation of two telocentric chromosomes, capable of lysing.

Univalents may occur in autotetraploids because of trivalents, as well as through the action of recessive genes for asynapsis or desynapsis. In the first case, the mean trivalent number per cell must correspond to the mean univalent number (34). In the second, the genes for asynapsis prevent chromosome conjugation, but due to the disbalanced process of chiasma terminalization (early chiasma sliding off a bivalent) disintegration of a bivalent into two univalents by diakinesis occurs (2, 4, 27, 30, 44).

Thus, along with cytogenetic, embryologic and morphophysiologic reasons, reduction of autotetraploid fertility may be due to genetic factors at molecular, cellular, organism and population levels.

Low fertility of experimental autotetraploids limits their practical use, which is why it is important to discover the causes of this phenomenon and to work out effective methods for increasing the fertility of new plant forms.

The purpose of this investigation was to discover the degree to which the decrease of fertility in autotetraploid buckwheat is connected with disturbances of meiosis, and to propose techniques for selection of high fertility plants.

Specifically, the aim was to discover: 1. The character of chromosome conjugation; 2. Chromosome behavior at the post- conjugation stages of meiosis in autotetraploid plants of buckwheat with different fertility; 3. The relationship of fertility to the appearance of multivalents and unbalanced gametes.

Material and methods

The material for this investigation comprised 3 populations of autotetraploid buckwheat. Population A was created on the basis of 40 elite plants of the autotetraploid buckwheat Bolshevik 4, which had passed through a 17-stage selection for increased fertility. The mean number of seeds per plant in this population was 137±9, which is 80.6% of the same index in the diploid variety Bolshevik. Population B was created on the basis of newly obtained autotetraploids of the same diploid variety Bolshevik, after the diploid had passed through a 12-stage selection for increased fertility. Generations C_1-C_2 were studied. The mean number of seeds per plant was 147±6, which is 86.5% of the fertility of diploids. Populations A and B are represented here as high fertile. Population C (low fertility) was formed on the basis of 66 low fertility plants, selected from autotetraploids and having 56±6 seeds per plant on the average at the initial stage of selection for low fertility. We also included in this

population progeny from 38 low fertility plants from recently obtained autotetraploids. As a whole, the mean number of seeds per plant in Population C was 29±3, which is 17.0% of diploid fertility, and 21.2 and 19.7% respectively, in relation to Populations A and B.

The fixation of buckwheat buds was conducted in the morning. The standard technique of temporal preparation was modified (61).

Results

The nature of chromosome conjugation was studied at diakinesis and Metaphase I. Table 1 presents data on the investigation of the nature of chromosome conjugation in plants of 3 populations of autotetraploid buckwheat. These data indicate that in Population A plants, which have been subjected to considerable breeding improvement, chromosomes form mainly bivalents (95.3%), with the formation of some quadrivalents (3.6%) and univalents (1.0%).

In Population B plants, in the first generations after colchicine treatment (C_1-C_2) we observed in general a bivalent type of chromosome conjugation (96.9%), with the formation of some quadrivalents (2.3%) and univalents (0.7%).

In the case of low fertility plants of Population C, we obtained the following data: 90.0% chromosomes formed bivalents, 3.4% - quadrivalents and 6.3% - univalents. There were many univalents in the five plants and the mean of the univalent configurations per PMC was 40.3, 23.2, 18.2, 15.6 and 15.0 respectively. There was observed, therefore, a reduction of chromosome conjugation with bivalents in low fertility plants of Population C at the cost of an increase of quadrivalents and, particularly, univalents, as compared with Populations A and B.

Table 2 shows multivalents (configurations including more than 4 chromosomes) and trivalents in PMCs in all 3 populations. The data indicate that Population B plants have much wider variation in the mean number of quadrivalents and bivalents than Population A plants, having comparatively more bivalents and fewer quadrivalents and univalents.

A much wider variation in the mean number of quadrivalents and, especially, of univalents, as compared to high fertility Populations A and B, is typical of low fertility plants of Population C. In terms of mean number of bivalents per one PMC, plants of Populations C and B had approximately the same indexes of variation. Population C plants differed significantly statistically from plants of Populations A and B in the mean number of quadrivalents, bivalents and univalents per one PMC.

To estimate the significance of the nature of chromosome conjugation of quadrivalents in plants of 3 populations of autotetraploid buckwheat, the obtained data were subjected to the χ^2 test (Table 3). The given data show that the nature of quadrivalent chromosome conjugation creates selection pressure as to fertility. It may therefore be concluded that quadrivalents play a particular role in reducing fertility in plants of autotetraploid buckwheat.

Using the χ^2 test, we analyzed the data on univalent frequency detected in buckwheat plants of 3 populations (Table 4). Univalents have a significant effect in decreasing the fertility of autotetraploid plants of buckwheat. The χ^2 test thus demonstrates the significant effect of quadrivalents and univalents in particular on the fertility of autotetraploids of buckwheat.

The effect of quadrivalents and univalents separately and their mutual effect in decreasing fertility of autotetraploids of buckwheat was evaluated by means of 2-factor analysis of variance (Table 5). Data of the analysis of variance demonstrated that the univalent effect on variation of fertility is 16.6% of total variation, and the quadrivalent effect only 2.0%. The combined effect of those two factors on variation in fertility was 22.1% of the sum of other factors affecting the fertility of buckwheat autotetraploids.

It can be hypothesized that disturbances in the process of chromosome conjugation,

Table 1. Nature of chromosome conjugation in plants of 3 populations of autotetraploid buckwheat.

Population	No of plants	Investigated PMCs	Chromosome associations					Mean number of chromosome associations per PMC				
			mv	IV	III	II	I	mv	IV	III	II	I
A	n = 15	472	1	136	2	7194	152	0.002	+0.288	+0.004	+15.242	+0.322
B	n = 26	795	-	147	5	12334	171	0.0	+0.185	+0.006	+15.514	+0.215
C	n = 16	345	-	94	4	4967	698	0.0	+0.273	+0.012	+14.400	+2.023

Table 2. Statistics of the mean number of different chromosome associations per PMC and their variation in plants of 3 populations of autotetraploid buckwheat.

Populations	Studied		Chromosome associations - $X \pm s_{\bar{X}}$		
	plants	PMC	quadrivalents	bivalents	univalents
A	15	472	0.25 ± 0.02	15.30 ± 0.01	0.39 ± 0.10
B	26	795	0.20 ± 0.03	15.47 ± 0.33	0.23 ± 0.07
C	16	345	0.31 ± 0.08	13.90 ± 0.43	2.88 ± 0.89

Note:
1. X - the mean value; $s_{\bar{X}}$ - mean error.
2. Student's test among different chromosome associations in plants of 3 populations. Symbols here are the following: d - difference among investigated groups; Sd - error of difference; t - test of significance; DF - degrees of freedom; p - significance.

Chromosome associations	Populations	d	Sd %	t	DF	p
quadrivalents	A - B	0.05	0.63	7.9	1265	>0.999
"	A - C	0.06	1.76	3.4	815	>0.999
"	B - C	0.11	1.82	6.0	1138	>0.999
bivalents	A - B	0.17	6.24	2.7	1265	0.99< <0.999
"	A - C	1.40	9.44	14.8	815	>0.999
"	B - C	1.57	11.05	14.2	1138	>0.999
univalents	A - B	0.16	2.25	7.1	1265	>0.999
"	A - C	2.49	19.15	13.0	815	>0.999
"	B - C	2.65	19.13	13.8	1138	>0.999

Table 3. Significance of effect of quadrivalent frequencies in PMC s of plants on autotetraploid buckwheat fertility (According to data in Table 1).

Populations	PMC with quadrivalents			Total	Significance
	1	2	3-8		
A	80 (72.0)	32 (31.0)	24 (32.0)	136 (136)	X^2 = 15.8 DF = 4
B	86 (78.0)	28 (33.0)	33 (35.0)	147 (147)	Table values X^2_1 = 9.5 (p = 0.95)
C	35 (50.0)	26 (21.0)	33 (22.0)	94 (94)	X^2_2 = 13.3 (p = 0.99) X^2_3 = 18.5 (p = 0.999)
Total:	201	96	90	377	0.99 < p < 0.999 Effect is significant.

Note:
1. According to the zero hypothesis, the absence of fertility effect on quadrivalent frequency, actual frequencies of cells with corresponding quadrivalent number should not differ from theoretical (written in brackets) and the X^2 value should not amount to the minimal value of significant differences - 9.5.
2. Cell frequencies with quadrivalent number more than 3 are combined and included in the same column.

Table 4. Significance of effect of the univalent frequency in PMCs of plants of autotetraploid buckwheat on fertility (According to data from Table 1).

Populations	PMC with univalents					Total	Significance
	1	2	3	4	5 - 32		
A	9 (9.0)	62 (35.0)	3 (6.0)	20 (24.0)	58 (78.0)	152 (152.0)	X^2 = 114.7 DF = 8
B	13 (10.0)	66 (39.0)	18 (7.0)	28 (27.0)	46 (87.0)	171 (171.0)	Table values: X^2_1 = 15.5 (p = 0.95)
C	39 (42.0)	108 (161.0)	21 (29.0)	112 (109.0)	418 (357.0)	698 (698.0)	X^2_2 = 20.1 (p = 0.99) X^2_3 = 26.1 (p = 0.999)
Total:	61	236	42	160	522	1021	p > 0.999 Effect is significant.

Note:
1. According to the zero hypothesis - the absence of effect, actual frequencies of cells with corresponding number of univalents should not differ from the theoretical (written in brackets) and the X^2 value should not amount to the minimal value of significant differences - 15.5.
2. Cell frequencies with univalent number more than 5 are combined and included in the same column.

Table 5. The effect of quadrivalent and univalent types of chromosome conjugation on the fertility of plants of autotetraploid buckwheat (data of analysis of variance)

Variation in data	Dispersion	Degree of freedom	Variance	Degree of effect	Fisher's test			
					fact	F - tabular		
						0.95	0.99	0.99
Total Y	1283.8	259	4.95	100.0				
Random Z	762.7	248	3.07	59.4				
Factorial X	521.1	11	47.36	40.6	15.4	1.8	2.3	3.1
Including:								
Factor A	25.3	2	12.65	2.0	4.1	3.0	4.7	7.2
Factor B	212.5	3	70.83	16.6	23.1	2.6	3.9	5.6
Gradiation combination of factors								
A + B	283.3	6	47.21	22.1	15.4	2.1	2.9	3.9

Note.
The sources of variation are represented as a 2-factor hierarchical dispersion complex for qualitative (alternatively variable) characters in the following order: factor A - variation for mean number of quadrivalents on PMCs in 3 populations of buckwheat; factor B - variation for mean number of univalents on PMCs. Resultant trait - grain number per investigated plant.

expressed in the formation of quadrivalents, univalents and separate multivalents, will cause disturbances in chromosome behavior at the post-conjugation stages of meiosis and, therefore, lead to formation of a definite number of unbalanced gametes, and subsequently zygotes. The next part of the paper is devoted to this problem.

As has been mentioned above, the nature of chromosome conjugation in 3 populations of autotetraploid buckwheat was studied at the stages of diakinesis and Metaphase I. We also investigated the form and behavior of different chromosome associations at the same meiotic stages.

Most bivalents in autotetraploid buckwheat were of the open type (Fig. 1). Quadrivalents were usually in the form of a ring (Fig. 2-3). However, these chromosome associations may also be in the form of a chain or a figure of eight (Fig. 2). Multivalents and trivalents (Fig. 2), as has already been said, occurred very seldom. At those stages of meiosis, there were PMCs which had univalents in addition to bivalents and quadrivalents (Fig. 4-5). The presence of univalents in the meiotic cells indicates partial or complete disturbance of chromosome conjugation. At the stage of diakinesis, we observed chromosome release from the terminal chiasma and at Metaphase I single chromosomes - univalents appeared (Fig. 6). This phenomenon was due to

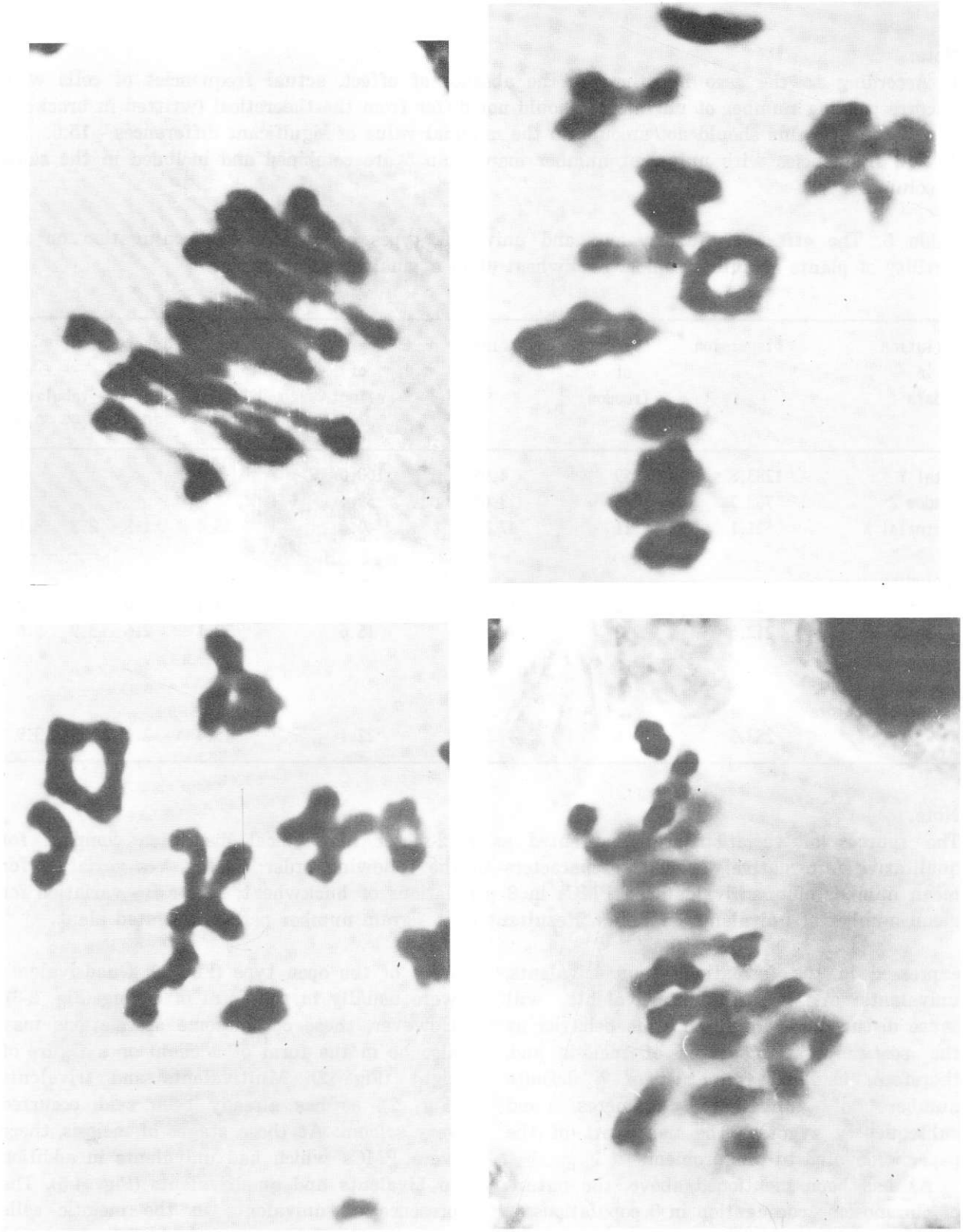
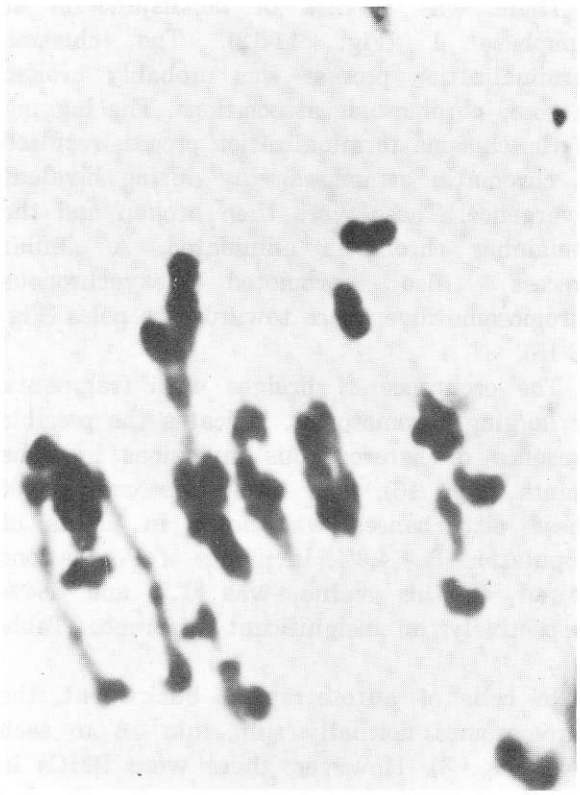


Fig. 1. Metaphase I; 16II. Different degrees of chiasma terminalization may be observed (x 3600).
Fig. 2. Metaphase I; 3IV+1III+8II+1I (x 3900).
Fig. 3. Metaphase I; 4IV+7II+2I. Quadrivalents: N1-◇ -4 chiasmata; N2---<-5 chiasmata; N3--< -3 chiasmata; N4>< -3 chiasmata (x 3750).
Fig. 4. Metaphase I; 13II+6I (x 3300).



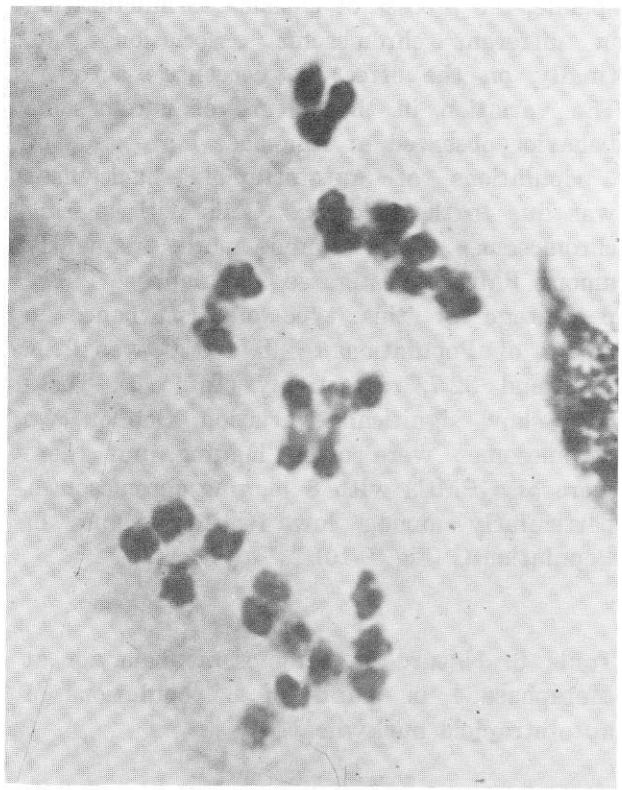


Fig. 5. Metaphase I; 10II+12I. Asynchronous process of chiasma terminalization in bivalents (x 3100).
Fig. 6. Metaphase I; Desynapsis. Chromosomes conjugating in the pachynema are repulsing each other and many of them are already lying side by side, not united by chiasmata, in the form of univalents (x 2800).

desynapsis. It should be noted that the desynaptic degree was different, the highest degree being expressed in most of the low fertility plants of Population C. At Metaphase I, we frequently observed a process of premature divergence, or vice versa, lagging divergence (the end of Metaphase I and the beginning of Anaphase I) of separate chromosome associations. This phenomenon is probably due to the neocentric activity of some chromosome segments, or to a disturbance in the process of chiasma terminalization. In the late Metaphase I we observed lagging in the process of chiasma terminalization and, as a result, chromatin strands were formed. In two plants of Population C, a process of complete chromatin degeneration occurred (Fig. 7). Those plants were sterile. It is likely that this phenomenon resembles in its phenotypic expression recessive mutation conditioning lack of meiosis in maize (46).

A general analysis of chromosome behavior at Metaphase I in plants of 3 populations of autotetraploid buckwheat is given in Table 6. The lowest disturbance percentage is observed in high fertility plants of Population B, which significantly exceeded that index in plants of Population A. The highest disturbance percentage is found in low fertility plants of Population C. The appropriate disjunction of homologues at Anaphase I depends on the nature of chromosome conjugation, the frequency and nature of chiasma distribution in different chromosome associations, the nature of the centromere coorientation which these associations take on the equator of the

spindle, the process of chiasma terminalization in different chromosome associations and, finally, on the effect of neocentric activity. The variation in the nature of chromosome behavior observed at Anaphase I in plants of 3 populations of autotetraploid buckwheat was due to these reasons. Cells with lagging chromosomes were found more frequently among PMCs investigated at Anaphase I. The percentage of this type of disturbance in plants of Populations A, B and C was 27.8, 12.2 and 32.7, respectively (Table 7). These data show significant variation. One or two chromosomes were often lagging, but there were also PMCs with 8 lagging chromosomes, particularly among low fertility plants of Population C (Fig. 9-10).

Table 6. Nature of chromosome behavior at Metaphase I in plants of 3 populations of autotetraploid buckwheat.

Populations	Investigated		
	plants	PMC total	including PMCs with disturbances
A	35	1009	260 (25.8)
B	26	795	171 (21.5)
C	21	452	220 (48.7)

Note. 1. The first figure in the last column is the absolute number, in brackets is the percent. 2. Student's test among populations. The symbols here are: d - difference among investigated groups; Sd - error of difference; t - test of significance; DF - degrees of freedom; p - significant difference.

Compared populations	d	Sd %	t	DF	p
A - B	0.04	2.0	2.0	1802	=0.95
A - C	0.23	2.8	8.1	1459	>0.999
B - C	0.27	3.2	8.5	1245	>0.999

There was a case of nondisjunction at Anaphase I (Fig. 11-12). The chiasma terminalization process was probably broken in some chromosome associations. The lagging of the chiasma terminalization process resulted in chromatin strands arising during bivalent divergence. These were then broken and the remaining chromatin eliminated. A similar process also promoted asynchronous chromosome divergence towards the poles (Fig. 13-15).

The occurence of bridges with fragments, or lagging chromosomes, indicates the possible presence of heterozygous inversions in some plants (Fig. 16). The highest percentage of these disturbances was noted in plants of Population B - 4.3%. In plants of Populations A and C this value was 1.7 and 1.4%, respectively, an insignificant difference (Table 7).

In cells of autotetraploid buckwheat, the chromosomes normally split into 16 to each pole (Fig. 8). However, there were PMCs in which 14 chromosomes went to one pole, and 18 to the other (Fig. 17) and even 12-20 chromosomes. There was one case in which 16 chromosomes went to one pole and 17 to the other (Fig. 15). The occurence of the extra chromosome in this case is likely to be a result of the premature divergence of a univalent in the first meiotic division. The lowest percentage of irregular divergence of chromosomes was found in plants of Population A - 0.6%, while in plants of Populations B and C the percentages were 2.8 and 3.0 respectively, not significantly different (Table 7).

In Anaphase I the highest percentage of changed PMCs was in Population C - 37.2%, and the lowest in Population B - 20.0%. Population A is intermediate - 30.5% of changed PMCs. The differences are significant (Table 7).

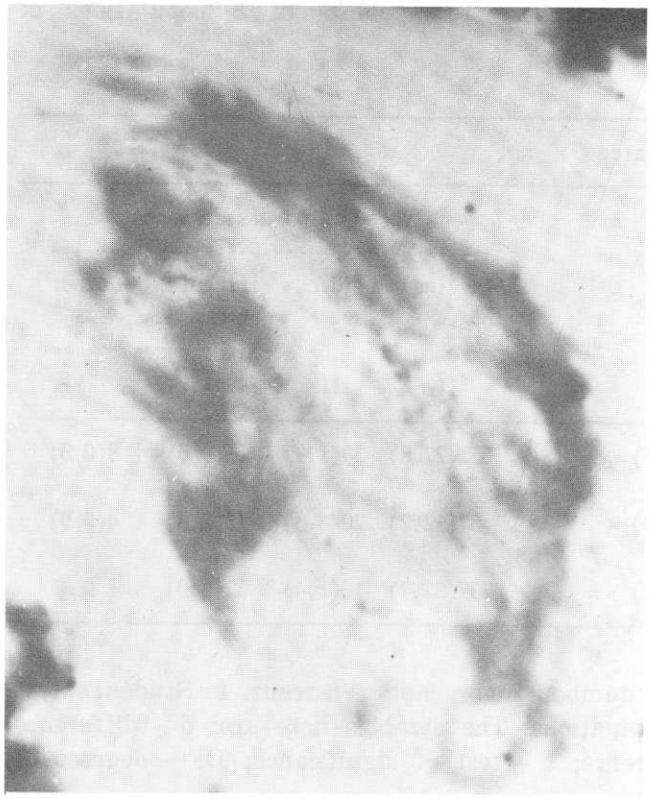
At the meiotic stage, 'Telophase I + Dyad', the most typical disturbances in PMCs of plants of all 3 populations were cells with micronuclei. These micronuclei probably originated from chromosomes lagging at Anaphase I. Data of the main types of disturbances are shown in Table 8.

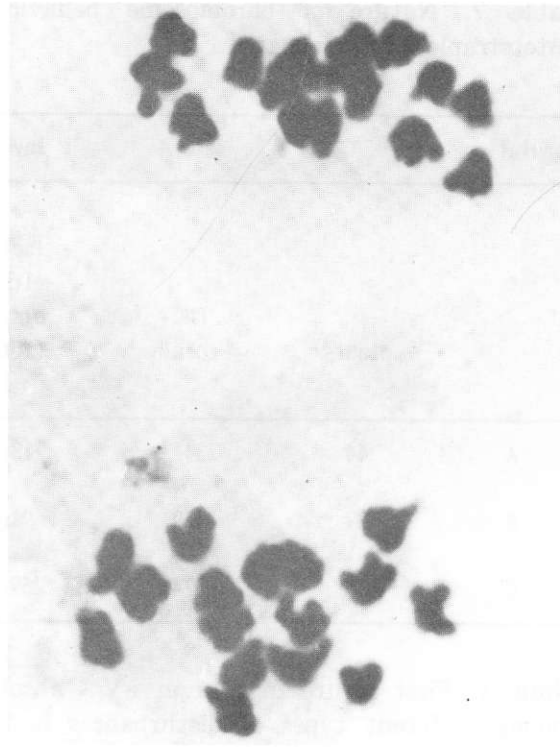
Table 7. Nature of chromosome behavior at Anaphase I in plants of 3 populations of autotetraploid buckwheat.

Population	Investigated						
				types of disturbances			
	plants	PMC total	including with disturb. (total) I	lagging of chromos. II	bridges III	irregular chromosome divergence IV	other V
A	44	2341	713(30.5)	651(27.8)	39(1.7)	15(0.6)	8(0.3)
B	27	469	94(20.0)	57(12.2)	20(4.3)	13(2.8)	4(0.9)
C	18	724	269(37.2)	237(32.7)	10(1.4)	22(3.0)	0

Note. 1. First figure in column I-V - absolute number, in brackets - percent. 2. Student's test among different types of disturbances in 3 populations. The symbols here are: d - difference among investigated groups; Sd - error of difference; t - test of significance; DF - degrees of freedom; p - significant difference.

Compared types of disturbances	Compared populations	d	Sd %	t	DF	p
I	A - B	0.11	1.97	5.6	2808	>0.999
	A - C	0.06	1.98	3.0	3063	0.99< <0.999
	B - C	0.17	2.45	6.9	1191	>0.999
II	A - B	0.08	1.64	4.9	2808	>0.999
	A - C	0.13	1.92	6.8	3063	>0.999
	B - C	0.21	2.24	9.4	1191	>0.999
III	A - B	0.026	0.98	2.7	2808	0.99< <0.999
	A - C	0.003	0.52	0.6	3063	0.95>
	B - C	0.029	1.05	2.8	1191	0.99< <0.999
IV	A - B	0.022	0.79	2.8	2808	0.99< <0.999
	A - C	0.024	0.66	3.6	3063	<0.999
	B - C	0.002	1.00	0.2	1191	0.95<





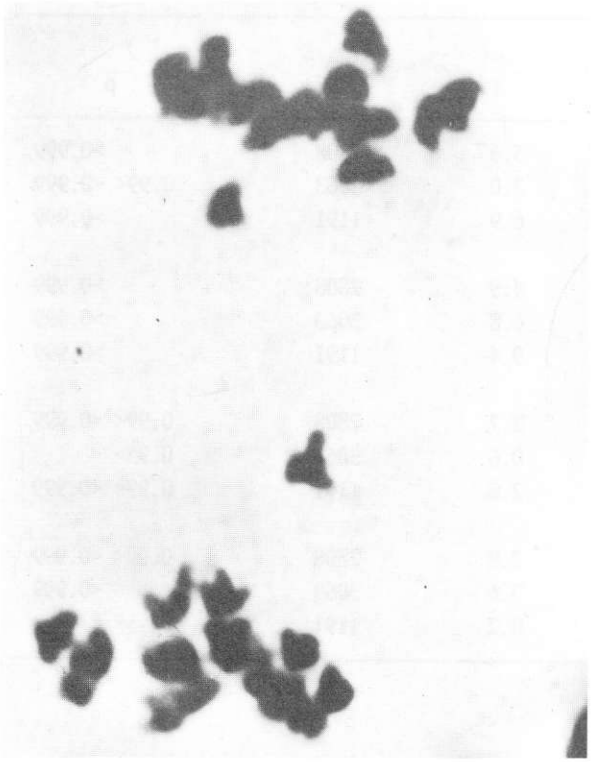


Fig. 7. Decay of the chromatin substance of a nucleus (x 3500).
Fig. 8. Anaphase I; Norm. (x 2600).
Fig. 9; Anaphase I; lagging of separate chromosomes (x 2600).

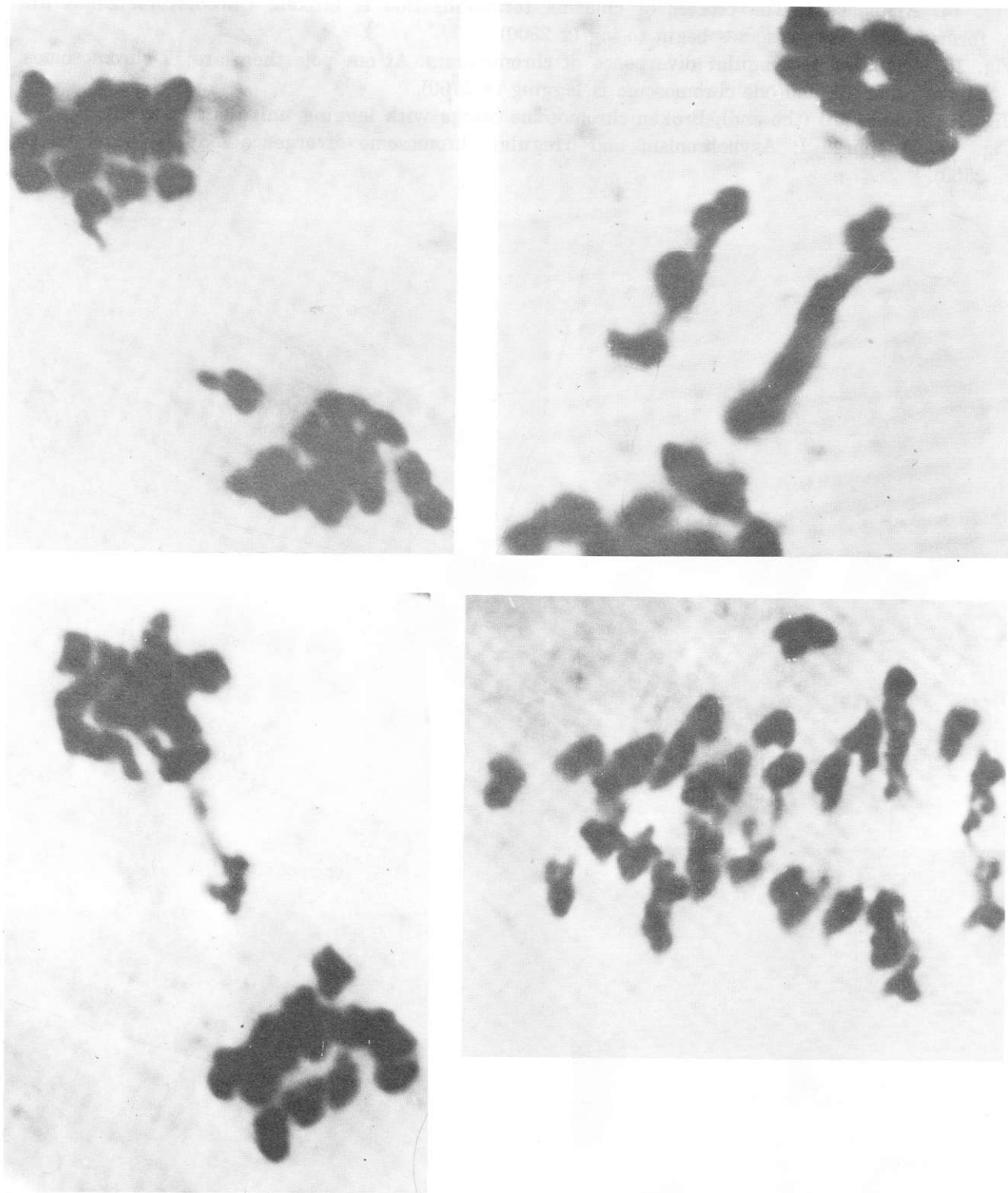
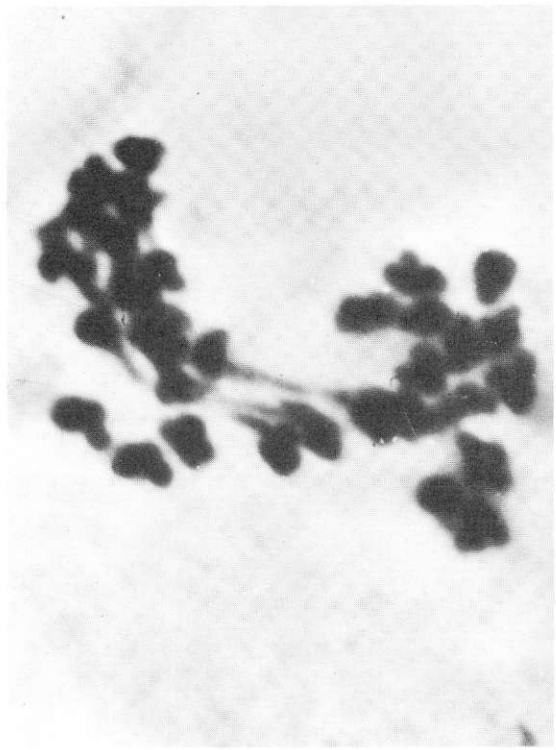
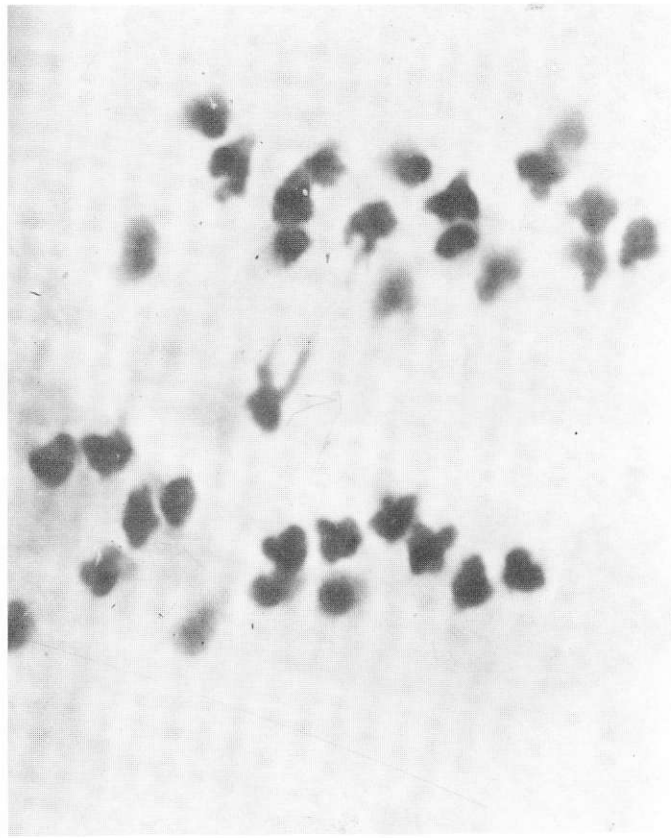
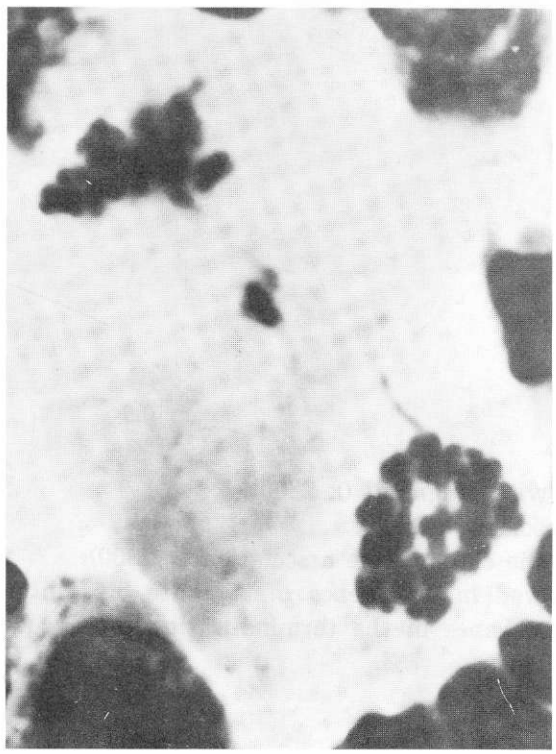


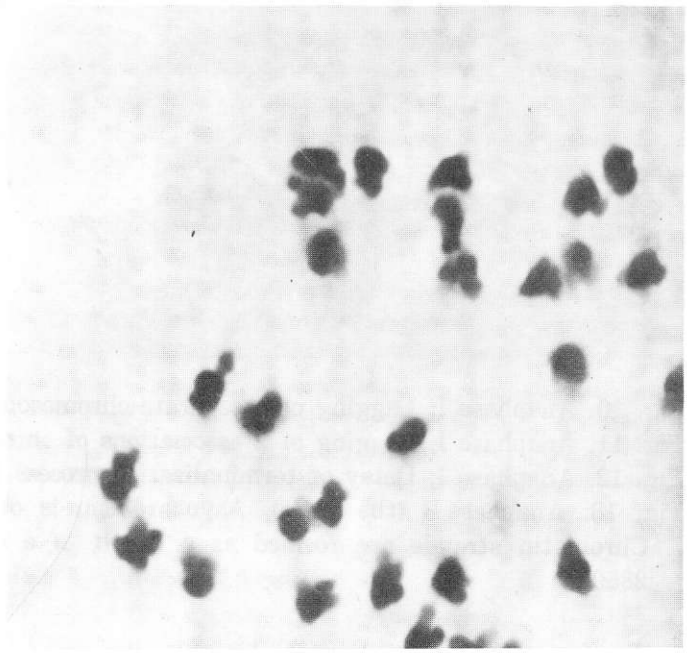
Fig. 10. Anaphase I; Lagging of a separate chromosome with fragment (x 2800).
Fig. 11. Anaphase I; Lagging of 2 associations of chromosomes (x 2800).
Fig. 12. Anaphase I; Delay of terminalization process in one chromosome association (x 2800).
Fig. 13. Anaphase I (the start); Asynchronism is observed in divergence of separate bivalents. Chromatin strands are formed as a result of a disturbance in the terminalization process (x 2850).

Fig. 14. Anaphase I; The process of chiasma terminalization is broken. Chromatin strands are formed and 2 chromosomes begin to lag (x 2800).
Fig. 15. Anaphase I. Irregular divergence of chromosomes. At one pole there are 17 chromosomes, at the other 15, and one chromosome is lagging (x 2700).
Fig. 16. Anaphase I (the end); Broken chromosome bridge with lagging univalent (x 2100).
Fig. 17. Anaphase I; Asynchronism and irregular chromosome divergence may be observed (x 2550).









Pollen mother cells with 2 micronuclei seldom occured. There was a similar percentage of cells with 2 micronuclei in plants of Populations A and C - 4.4 and 4.3% respectively, (the difference is not significant). In Population B, the figure was 1.6%, significantly different from that of Populations A and C).

PMCs with 3 micronuclei occured very seldom. The difference among populations was not significant.

In the meiotic stage, 'Telophase I + Dyad', the highest percentage of changed pollen mother cells was thus in low fertility plants of Population C - 24.3% and the lowest - 9.6% in plants of Population B. Plants of Population A had 20.7% of disturbances. The difference among these data is significant

Table 8. Nature of chromosome behavior at Telophase I + Dyad in plants of 3 populations of autotetraploid buckwheat.

Popul.	Investigated					
			incl. with disturb. (total)	types of disturbances		
	plants	PMCs total	I	PMC with 1 micronucl. II	PMC with 2 micronucl. III	PMC with 3 micronucl. IV
A	23	540	112 (20.7)	83 (15.4)	24 (4.4)	5 (0.9)
B	31	1227	118 (9.6)	95 (7.7)	19 (1.6)	4 (0.3)
C	14	259	63 (24.3)	49 (18.9)	11 (4.3)	3 (1.2)

Notes as in Table 7.

Compared types of disturbances	Compared populations	d	Sd %	t	DF	p
I	A - B	0.114	1.92	5.9	1765	>0.999
	A - C	0.03	3.16	0.9	797	0.95>
	B - C	0.144	2.78	5.2	1484	>0.999
II	A - B	0.07	1.61	4.3	1765	>0.999
	A - C	0.04	2.83	1.4	797	0.95>
	B - C	0.11	2.57	4.3	1484	>0.999
III	A - B	0.024	0.89	2.7	1765	0.99< <0.999
	A - C	0.001	1.18	0.1	797	0.95>
	B - C	0.024	0.89	2.7	1484	0.99< <0.999
IV	A - B	0.006	0.47	1.3	1765	0.95>
	A - C	0.0026	0.84	0.3	797	0.95>
	B - C	0.0086	0.72	1.2	1484	0.95>

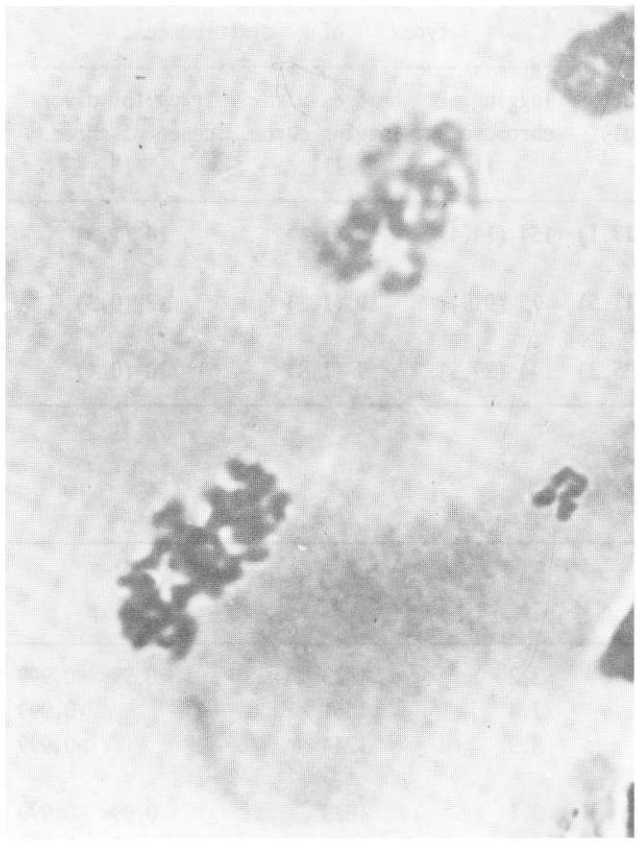
Table 9. Nature of chromosome behavior at Metaphase II in plants of 3 populations of autotetraploid buckwheat.

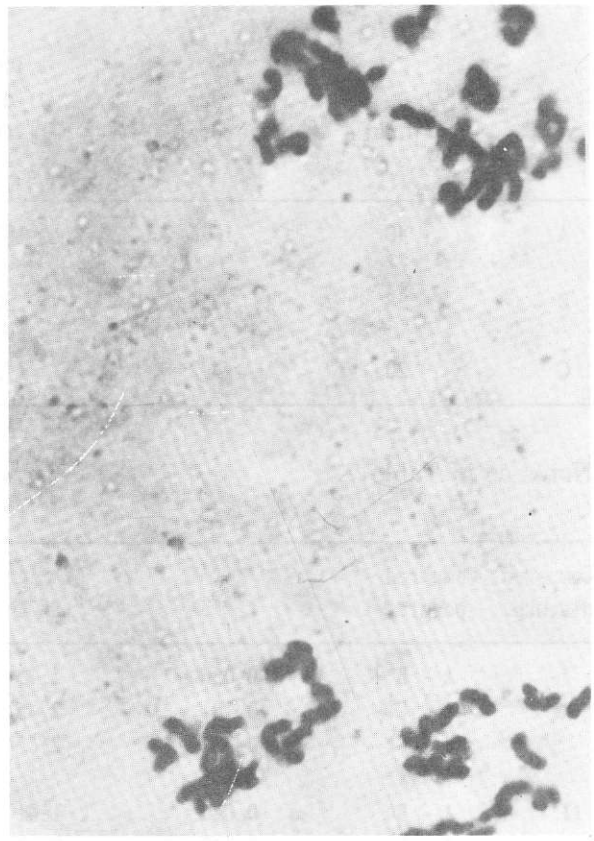
Popul.	Investigated							
			types of disturbances					
				chromosomes discarded				
	plants	PMCs total	incl. wit disturb. (total) I	1 II	2 III	4 IV	asynchron. divergence of chromosome V	other
A	28	511	105 (20.5)	53 (10.4)	27 (5.3)	16 (3.1)	3 (0.6)	6 (1.2)
B	28	593	63 (10.6)	33 (5.6)	17 (2.9)	5 (0.8)	2 (0.3)	6 (1.0)
C	15	221	59 (26.7)	26 (11.8)	15 (6.8)	8 (3.6)	10 (4.5)	0

Notes as in Table 7.

Compared disturb.	Compared populations	d	Sd %	t	DF	p
I	A - B	0.10	2.24	4.5	1102	>0.999
	A - C	0.06	3.46	1.7	730	0.95>
	B - C	0.16	3.32	4.8	812	>0.999
II	A - B	0.048	1.70	2.8	1102	0.99< <0.999
	A - C	0.016	2.65	0.6	730	0.95>
	B - C	0.064	2.43	2.6	812	=0.99
III	A - B	0.024	1.22	2.0	1102	=0.95
	A - C	0.015	2.00	0.7	730	0.95>
	B - C	0.039	1.87	2.1	812	0.95< <0.99
IV	A - B	0.022	0.77	2.9	11 02	0.99< <0.999
	A - C	0.006	1.58	0.4	730	0.95>
	B - C	0.028	1.45	1.9	812	0.95>
V	A - B	0.003	0.39	0.8	1102	0.95>
	A - C	0.039	1.45	2.7	730	0.99< <0.999
	B - C	0.042	1.43	2.9	812	0.99< <0.999

Fig. 18. Metaphase II; Divergence of chromatids of the univalent, which have lagged on the equator at 1st division during coorientation (x 1550).
Fig. 19. The end of Metaphase II and the start of Anaphase II; asynchronism in divergence of chromosomes to poles (x 1900).





(Table 8).

Table 9 shows the main types of disturbances observed in PMCs of plants of 3 populations at Metaphase II. At that stage we most often observed separate chromosomes distributed outside the equatorial plates (fig. 18). That type of disturbance is caused by lagging of chromosomes at Anaphase I and is revealed by micronuclei at the 'Telophase I + Dyad' stage.

While investigating that meiotic stage, PMCs with asynchronous chromosome divergence were found, i.e., at one pole chromosomes were already entering Anaphase II, but at the other pole this process had not been started and chromosomes were at Metaphase II (Fig. 19). This was observed most frequently in PMCs of Population C plants.

At Metaphase II, the highest percentage of changed PCMs was thus found in low fertility plants of Population C - 26.7%, and the lowest - 10.6% - in high fertility plants of Population B. High fertility plants of Population A demonstrated 20.5% of disturbances. The difference between Populations A and C is not significant, but between B and the other two it is.

The comparative characteristics of the main types of disturbances revealed in PMCs in

Table 10. Nature of chromosome behavior at Anaphase II in plants of 3 populations of autotetraploid buckwheat.

Popul.	Investigated					
				types of disturbances		
	plants	PMCs total	incl. with disturb. (total) I	lagging of chromosomes II	bridges with lagging chrom. III	irregular divergence of chrom. IV
A	40	1071	183 (17.1)	154 (14.4)	15 (1.4)	14 (1.3)
B	28	754	87 (11.5)	75 (9.9)	10 (1.3)	2 (0.3)
C	20	492	173 (35.2)	144 (29.3)	9 (1.8)	20 (0.4)

Notes as in Table 7.

Compared disturb.	Compared popul.	d	Sd %	t	DF	p
I	A - B	0.056	1.73	3.2	1823	0.99< <0.999
	A - C	0.181	2.45	7.4	1561	>0.999
	B - C	0.237	2.65	8.9	1244	>0.999
II	A - B	0.044	1.41	3.1	1823	0.99< <0.999
	A - C	0.149	2.24	6.6	1561	>0.999
	B - C	0.193	2.24	8.6	1244	>0.999
III	A - B	0.003	0.44	0.2	1823	0.95>
	A - C	0.004	0.71	1.1	1561	0.95>
	B - C	0.005	0.71	1.1	1244	0.95>
IV	A - B	0.010	0.37	2.7	1823	0.99< <0.999
	A - C	0.028	0.95	2.9	1561	0.99< <0.999
	B - C	0.038	0.92	4.1	1244	>0.999

plants of 3 populations of autotetraploid buckwheat at Anaphase II is given in Table 10. It may be seen that at this meiotic stage, one of the most frequently occurring disturbances was the lagging of chromosomes during their divergence towards the poles (Fig. 20).

At the meiotic stage Anaphase II, PMCs were also found in which chromosomal bridges and bridges with fragments were observed. The number of cells with that type of disturbance was not very large, 1.4, 1.3 and 1.8%, in Populations A, B and C respectively. The difference among the populations is not

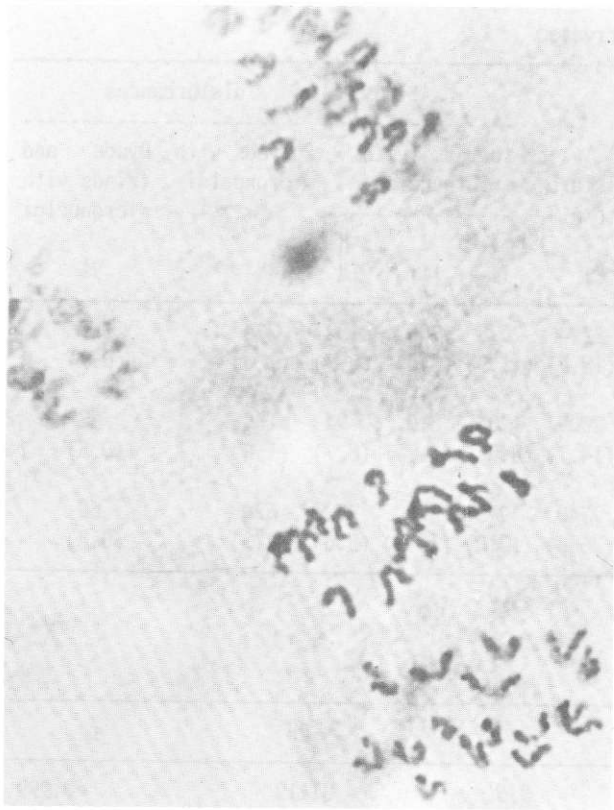
Table 11. Nature of chromosome behavior at Telophase II + Tetrad in plants of 3 populations of autotetraploid buckwheat.

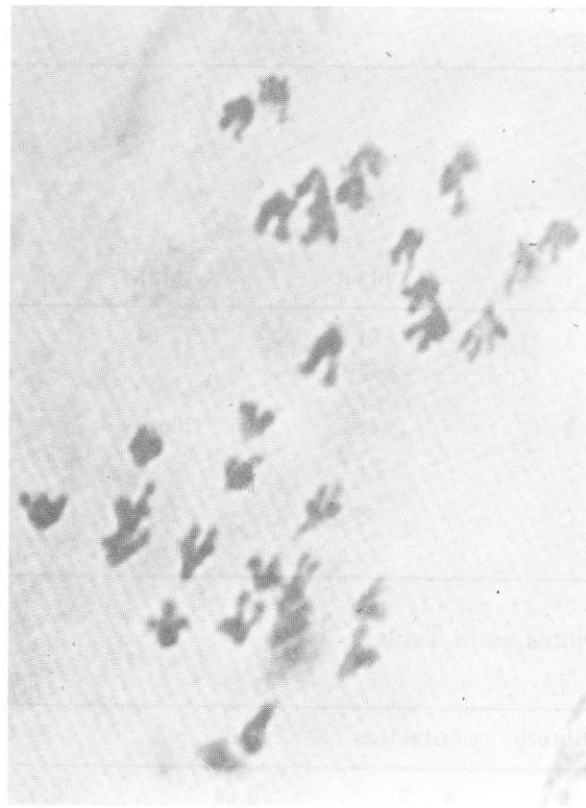
Popul.	Investigated							
				types of disturbances				
			incl. with disturb. (total)	tetrads with micronuclei			Polyads with micronuclei	Dyads and triads with micronuclei
				1	2	3-8		
	plants	PMCs total	I	II	III	IV	V	VI
A	91	17504	3304 (18.9)	2078 (11.9)	700 (4.0)	132 (0.7)	274 (1.6)	119 (0.7)
B	66	13610	2009 (14.7)	1204 (8.8)	510 (3.7)	94 (0.7)	136 (1.0)	68 (0.5)
C	55	7496	2740 (36.6)	973 (13.0)	815 (10.9)	272 (3.6)	620 (8.3)	60 (0.8)

Notes as in Table 7.

disturb.	populations	d	Sd %	t	DF	p
I	A - B	0.04	0.42	9.5	31112	>0.999
	A - C	0.18	0.62	29.0	24998	>0.999
	B - C	0.22	0.62	35.5	21104	>0.999
II	A - B	0.030	0.35	8.6	31112	>0.999
	A - C	0.011	0.40	2.7	24998	0.99< <0.999
	B - C	0.041	0.40	10.3	21104	>0.999
III	A - B	0.002	0.22	0.9	31112	0.95>
	A - C	0.069	0.35	19.7	24998	>0.999
	B - C	0.071	0.36	19.7	21104	>0.999
IV	A - B	0.0005	0.09	0.6	31112	0.95>
	A - C	0.0285	0.23	12.4	24998	>0.999
	B - C	0.0290	0.23	12.6	21104	>0.999
V	A - B	0.006	0.13	4.6	31112	>0.999
	A - C	0.067	0.33	20.3	24998	>0.999
	B - C	0.073	0.33	22.1	21104	>0.999
VI	A - B	0.002	0.09	2.2	31112	0.95< <0.99
	A - C	0.001	0.12	0.8	24998	0.95>
	B - C	0.003	0.12	2.5	21104	0.95< <0.99

Fig. 20. Anaphase II; Norm. (x 1500).
Fig. 21. Anaphase II; One of two divisions with irregular chromosome divergence to poles (14-I-17) (x 2900).





significant.

At that meiotic stage we also observed cells with irregular divergence of chromosomes to the poles. For example, Fig. 26 shows one of the poles at Anaphase II with chromosome divergence of 14-1-17. The highest percentage of PMCs with a similar disturbance was observed in plants of Population A - 1.3%. The percentage of similar PMCs was 0.3 and 0.4 respectively in plants of Populations B and C. The difference between Population A and Populations B and C is significant.

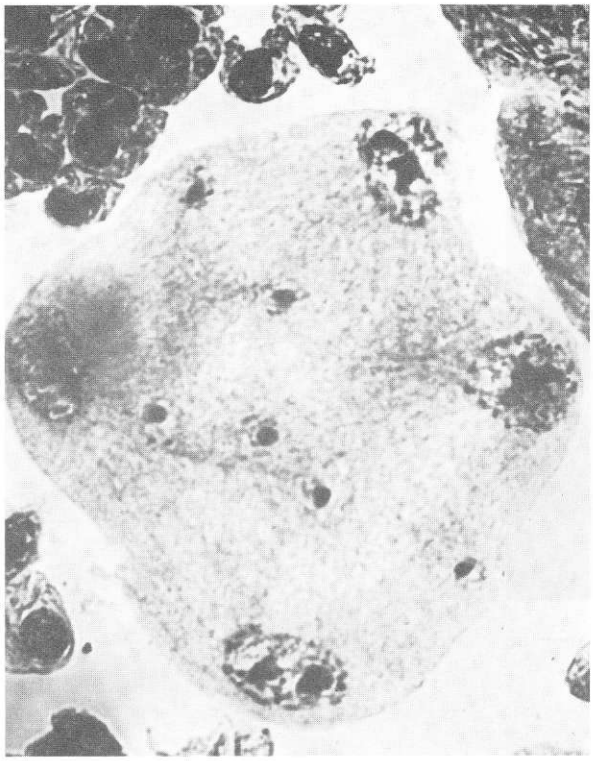
A comparative description of the main types of disturbances, revealed in PMCs of plants in 3 populations of autotetraploid buckwheat at the final meiotic stage 'Telophase II + Tetrad' is given in Table 11. It is clear from this table that at this meiotic stage, cells with micronuclei often occurred (Fig. 22). Tetrads with one micronucleus (Fig. 24) were most common and cases with 2 micronuclei were rare. The highest percent of tetrads with one micronucleus was found in plants of Population C - 13.0%, in Population A the figure was 11.9% and in Population C 8.8%. The difference among the populations is significant.

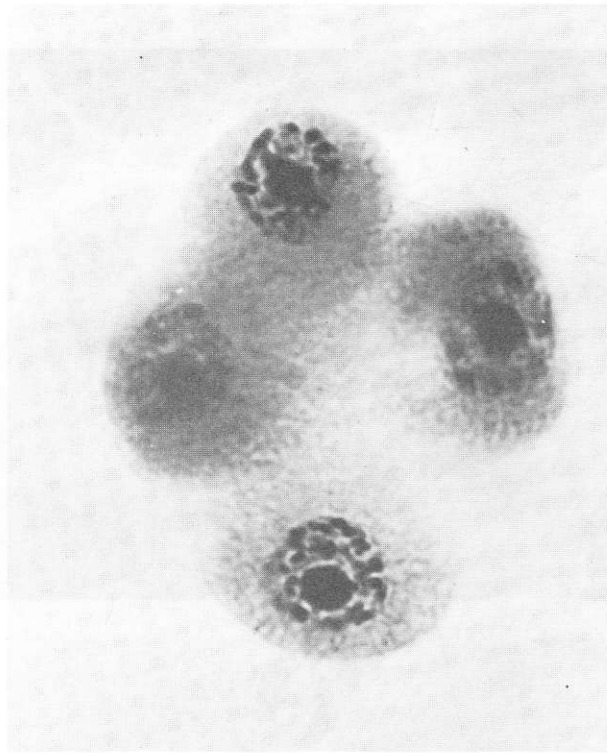
Tetrads with micronuclei from 3 to 8 were about 5 times as frequent in plants of Population C as in Populations A and B. The percentage of such cells was the same (0.7%) in high fertility Populations A and B.

An increase in the amount of polyads with micronuclei (Fig. 25-26) was typical of low fertility plants of Population C in comparison with high fertility plants of Populations A and B.

At the meiotic stage 'Telophase II + Tetrad' we also observed dyads and triads with micronuclei.

In the post-conjugation meiotic stages of autotetraploid buckwheat, the main types of disturbances were: PMCs with lagging





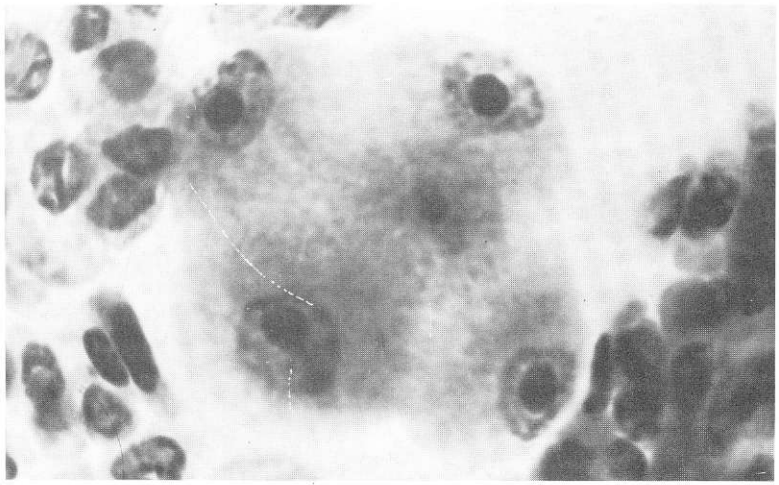
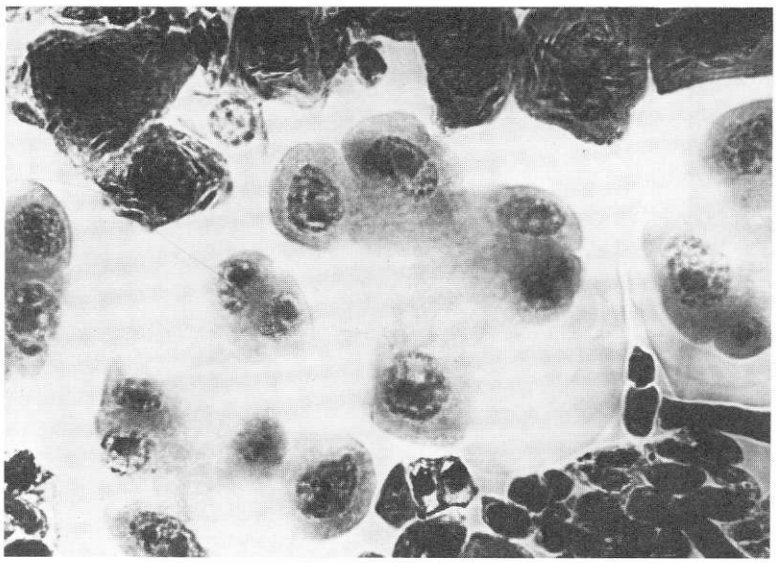
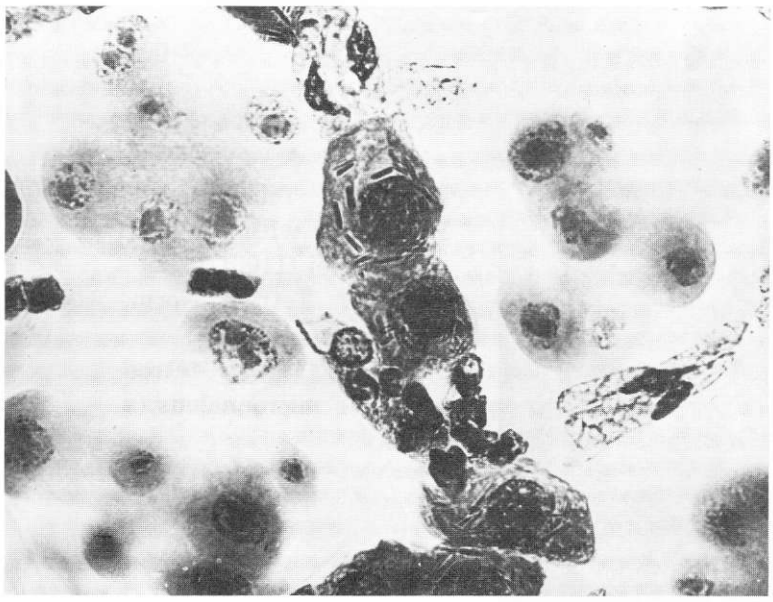


Fig. 22. Telophase II; PMC with six micronuclei (1100).
Fig. 23. Tetrad; Norm. (x 1400).
Fig. 24. 4+1, tetrad with one micronucleus (x 1250).

chromosomes at Anaphase I; PMCs with micronuclei at Telophase I + Dyad; PMCs with disorientated chromosomes at Metaphase II; PMCs with lagging chromosomes at Anaphase II and finally, PMCs with micronuclei at Telophase II + Tetrad. It follows that it is probably precisely these types of disturbances which are primarily responsible for the formation of non-balanced gametes, and, hence, the reduction of fertility in plants of autotetraploid buckwheat, and since the range of the main types of disturbances is similar in both high and low fertility plants, so there are common mechanisms leading to the reduction in fertility of plants of autotetraploid buckwheat. As has already been shown, such mechanisms affecting fertility are: 1)

Fig. 25-26. Polyads (x 850).

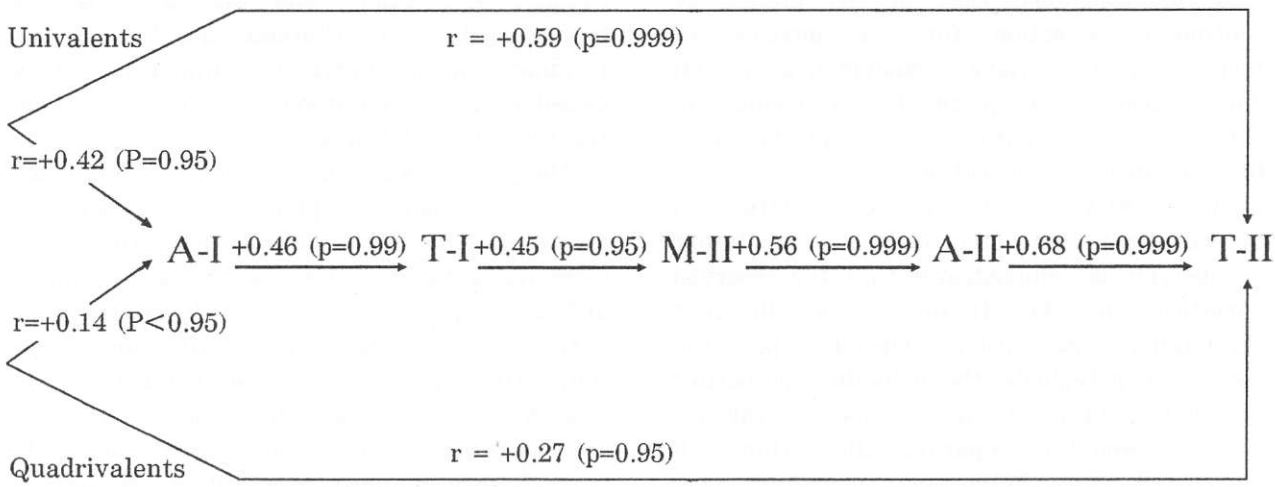




disturbances in the process of normal chromosomal conjugation, and 2) quadrivalent type of chromosome conjugation.

The question arises as to whether there is a correlation between quadrivalents - univalents revealed by us at the stages of diakinesis and Metaphase I, and the main types of disturbance at the post-conjugation stages of meiosis. To answer this question, we established correlation coefficients between the number of quadrivalents and univalents, and the number of main types of disturbances detected at the post-conjugation stages of meiosis in plants of autotetraploid buckwheat. The obtained data are presented in Fig. 27. From the data it may be seen that in all cases except one, the values of the correlation coefficients were positive and significant. The correlation coefficient between the quadrivalent number and PMC's number with

Fig. 27. Correlation between the number of quadrivalents and univalents and the number of the main types of disturbance at post-conjugation stages of meiosis in plants of autotetraploid buckwheat.



Note. Coefficients of correlation were established with the following types of disturbance: Anaphase I - the lagging of chromosomes; telophase I + dyad - MPSs with micronuclei; Metaphase II - disoriented chromosomes; Anaphase II - lagging of chromosomes; Telophase II + Tetrad - PMCs with micronuclei.

disturbances at Anaphase I was the lowest and was not significant. The obtained data allowed us to conclude that there is a logical connection between conjugation processes, chromosome behavior at the post-conjugation stages of meiosis and the formation of unbalanced gametes, affecting directly the fertility of plants of autotetraploid buckwheat.

Discussion

An analysis of data obtained during investigation of the nature of conjugation and chromosome behavior at the post-conjugation stages of meiosis in relation to plant fertility in autotetraploid buckwheat, shows that the main contribution to the formation of unbalanced gametes and, therefore, to the reduction of fertility is from univalents (16.6%). A quadrivalent type of chromosome conjugation also leads to the formation of unbalanced gametes. However, the contribution of this association to the reduction of fertility in autotetraploid buckwheat is about 8 times less (2.0%), than that of univalents. A positive correlation between quadrivalents-univalents and the main types of disturbances in the 1st and 2nd meiotic division was also found. The fertility of plants of autotetraploid buckwheat thus depends upon the nature of chromosome conjugation and the formation of unbalanced gametes. The presence of a wide variation in the number of chromosome associations in PMCs (quadrivalents and univalents) allows the conclusion that the genotype affects the nature of chromosome conjugation, the number of chiasmata, the formation of quadrivalents and, finally, the mechanism of univalent formation, that in total it conditions the nature of chromosome behavior in the 1st and 2nd meiotic divisions and, hence, the higher or lower fertility of autotetraploid buckwheat.

It should also be noted that the meiosis of autotetraploids has been studied during the first years after they were obtained (18-19).

The authors of that investigation noted that "beside quadrivalents there were a number of bivalents; trivalents and univalents occured as an exception". As a matter of experience it may be concluded that in the process of continuous selection for the increase of fertility of the variety Bolshevik-4, a gradual diploidization took place, i.e. a change in chromosome conjugation from quadrivalent to bivalent in later generations.

An analysis of literary data on cytogenetical investigation of different autopolyploids demonstrates that the observed variation in the frequency of different chromosome associations depends upon the species of polyploids, the individual properties of some plants within the species, varietal character and even separate cells within each plant (19, 32). Polyploids possessing long chromosomes form multivalents more often than those with short chromosomes (12, 29). It is evident, too, that multivalent frequency depends also on chiasma frequency, and it may be proportional to chromosome length (12, 21, 36, 47). In other words, longer chromosomes have a large chiasma frequency and form multivalents more often, including also quadrivalents, and vice versa. Thus, different associations per cell in autopolyploids depend on variations in chiasma frequency. Over and above, the comparative study of chiasma frequency in diploids and autotetraploids obtained from them has shown that, as a rule, the relative chiasma frequency is not changed during transposition to the polyploid level (1, 21, 32-33).

From this point of view, data from the karyological study of diploid buckwheat carried out by V.V. Mansurova are of interest (31). In diploid buckwheat, chromosome N1 is the longest, i.e. 20.2 units, chromosomes N2 and N3 are 17.32 and 17.12 units respectively. The shortest chromosomes are N7 and N8, with lengths of 13.15 and 11.26 units respectively. Chromosomes N4 - N6 are intermediate with corresponding lengths of 15.84, 15.43 and 15.07 units.

When studying the nature of chromosome conjugation in diploid buckwheat we found that, as a rule, 3 bivalents presented a closed type (2-3 chiasmata), and five - an open type (1 chiasma; Fig. 28). Hence it may be concluded that the longest chromosome, N1, participates in the formation of quadrivalents in autotetraploid buckwheat and of other complex associations and the same part is probably played by Chromosomes N2 and N3, forming during bivalent conjugation of a closed type. Short chromosomes may condition the formation of univalents.

The variation in chiasma frequency between separate plants is genetically controlled (27, 47). In addition, there are recessive genetic factors (genes of asynapsis and desynapsis), characterized by a polygene nature of inheritance affecting the conjugation process and, in particular, the process of chiasma formation. It was demonstrated in our study that univalents were formed in most cases due to premature chiasma terminalization of separate bivalents at a late prophase of meiosis, i.e., as a result of desynapsis.

While analysing the fertility of experimental autotetraploid of buckwheat derived from 9 diploid varieties, V. V. Sakharov et al. arrived at an important conclusion on the great role of the initial diploid material involved in polyploidization. They wrote: "Tetraploid forms with sharply reduced fertility originate from hereditarily defective diploid material, on the basis of which polyploidy cannot give good effect" (51).

Our data on newly obtained autotetraploids of buckwheat (Population B) fully confirm this conclusion. In PMCs of plants of Population B, even in C_2 we observed a mainly bivalent type of chromosome conjugation. The plants of that population were characterized by a lower percentage of changed PMCs at the post-conjugation stages of meiosis as compared with plants of high fertility Population A, further along the path of selective improvement. In addition, plants of Population B were not more fertile than plants of Population A (62). The principal cause may lie in the initial diploid material. As stated above, for obtaining new autotetraploids of buckwheat (Pop. B) we used plants of the diploid buckwheat variety 'Bolshevik' which had passed through continuous selection for fertility improvement.

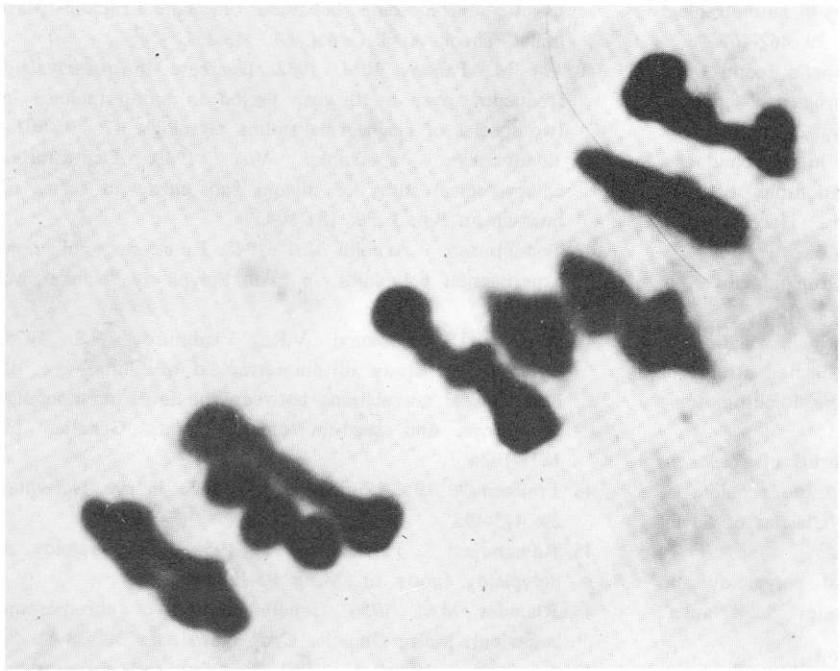


Fig. 28. Metaphase I (diploids) (x 4100).

In the creation of new autopolyploids it is therefore necessary to study the initial diploid material by the whole complex of agronomic characters, and then to use the best plants for colchicine treatment and transposition to the polyploid level.

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До проблеми плодовитості аутотетраплоідної гречки

Повідомлення I. Мейоз та плодовитість експериментальних аутотетралоідів *Fagopyrum esculentum Moench*

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Резюме

Дослідження проведені на рослинах трьох популяцій аутотетраплоідної (4n) гречки. Популяція А представлена рослинами, що були створені В. В. Сахаровим та його співробітниками у 1941 р. з диплоідного (2n) сорту "Більшовик", які пройшли до початку роботи 17-разовий відбір на плодовитість (сорт "Більшовик-4"). Популяція В представлена рослинами C_1–C_2, що були створені з того ж 2n сорту "Більшовик", які перед поліплоідізаціею пройшли 12-разовий відбир на плодовитість. Популяція С створена з низькоплодовитих рослин популяцій А та В. Плодовитість рослин популяції А складала 80,6%, В – 86,5% і С – 20,0% по відношенню до 2n. Встановлено, що у рослин популяцій А та В 95–97% хромосом, а у С – 90% кон "югували бівалентами. Велика мінливість хромосомних асоціацій в МКП показує, що характер кон"югації хромосом, кі лькість хіазм та іх терміналізація, поведінка хромосом в першому та другому поділі мейозу генетично детерміновані. Унівалснти в своій більшості утворювались внаслідок передчасної терміналізації хіазм в окремих бівалентах. Вказано на зв"язок квадривалентів та унівалентів з поведінкою хромосом на постскон-"югаційних стадіях, вплив цих асоціацій на процес утворення незбалансованих гамет і в кінці кінців на плодовитість 4n гречки. Створені нами рослини популяції В з 2n, що витримали довготривалий відбір на плодовитість, не поступались популяції А як по мейозу, так і плодовитості, що дозволяє зробити висновок про велику роль генотипу вихідних диплоідів, залучуваних в поліплоідізацію.

К проблеме плодовитости аутотетраплоидной гречихи

Сообщение I. Мейоз и плодовитость экспериментальных аутотетралоидов *Fagopyrum esculentum Moench*

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Резюме

Исследования проведены на растениях трех популяций аутотетраплоидной (4n) гречихи. Популяция А представлена растениями, полученными В. В. Сахаровым с сотрудниками в 1941 г. из диплоидного (2n) сорта Большевик и прошедшими к началу работы 17-кратный отбор на плодовитость (сорт Бльшевик-4). Популяция В представлена растениями C_1–C_2, полученными из того же (2n) сорта Большевик, но перед полиплоидизацией прошедшими 12-кратный отвор на плодовитость. Популяция С создана из низкоплодовитых растений популяций А и В. Плодовитость растений популяции А составляла 80,6%, В – 86,5% и С – 20% по отношению к 2n. Установлено, что у растений популяций А и В 95–97% хромосом, а у С – 90% конъюгировали бивалентами. Большая изменчивость хромосомных ассоциаций в МКП показывает, что характер конъюгации хромосом, количество хиазм и их терминализация, поведение хромосом в первом и втором делениях мейоза генетически детерминированы. Униваленты в своем большинстве образовывались следствие преждевременной терминализации хиазм у отдельных бивалентов. Показана связь квадривалентов и унивалентов с поведением хромосом на постконъюгационных стадиях, влияние этих ассоциаций на процесс образования несбалансированных гамет и в итоге на плодовитость 4n гречихи. Вновь созданные нами растения популяции В, полученные из 2n, прошедшие длительный отбор на плодовитость, не уступали популяции А по мейозу и плодовитости. Это позволяет сделать вывод о большой роли генотипа исходных диплоидов, вовлекаемых в полиплоидизацию.

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ВРЕДИТЕЛИ ГРЕЧИХИ И ФАКТОРЫ ВЛИЯЮЩИЕ НА ИХ ЧИСЛЕННОСТЬ

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В работе представлены данные о биологии наиболее распространенных вредителей гречихи, рассматриваются факторы влияющие на их численность.

Введение. Известно, что мировые потери урожая сельскохозяйственных культур достигают примерно 1/3 их материального валового сбора и выражаются в 750 млрд. долларов (М. Д. Вронских, 1981).

Защита растений гречихи от вредных патогенов является важным резервом повышения её урожайности. Видовой состав вредителей гречихи их биология и вредоносность изучены недостаточно и пока имеютсся лишь неполные данные о насекомых, которые вредят этой культуре. Их насчитывается более 40 видов.

В. Г. Плечинский (1928) на территории РСФСР в посевах гречихи обнаружил 16 видов вредителей. И. Н. Елагин (1962) отмечает, что посевы гречихи в Курской области посещают более 20 видов вредителей.

Обследование посевов гречихи в Пермской области показало наличие 22 вредителей (З. П. Журавлева и др. 1979, 1982).

А. С. Кротов (1976) указывает, что на гречихе встречается 11 видов нематод.

Специализированными вредителями гречихи считают гречишную блоху, гречишного комарика, гречишного долгоносика, гречишную листоблошку и др.

З. П. Журавлева и др. (1972) установили, что потери урожая зерна гречихи от вредителей составляют 2,5 ц/га.

Материал и методы. Для изучения видового состава и численности вредителей обследовали селекционные посевы Проблемной научно-исследовательской лаборатории по гречихе Каменец-Подольского сельскохозяйственного института, образцы из коллекции ВИР, дикие сородичи, агротехнические опыты и участки размножения новых и районированных сортов.

Сбор насекомых проводили через каждые 10 дней энтомологическим сачком на протяжении всего вегетационного периода. При изучении морфобиологических признаков гречихи пользовались бинокулярной лупой. Содержание каротина и клетчатки определяли по методике Кимаковского В. И. и др. (1988).

Результаты и обсуждение. Исследования показали, что в посевах гречихи встречаются следующие вредители: отряд Heteroptera сем. Anthocoridae вид Anthocoris nemoralis – хищный клоп; отряд Homoptera – подотряд Cicadinea Цикадовые, сем. Носатки – Dictyopharidae вид Dictyophara europea L.; сем. Cicadellidae – Цикаделиевые, вид Cicadella viridis – цикадка зеленая; сем. Aphrophoridae – Пенницы, виды Philaenus spumarius L., Aphrphora alni; Fall. сем. Jassidae – Цикадки, виды-цикадка волнистая-Platymetopius undatus Deg, цикадка полосатая Psammotettix striatus L., цикадка шеститочечная Macrosteles sesenotatus Fale. Подотряд Psyllinea – псиплиды, или листоблошки.

Наиболее распространенными и вредоносными были гречишная или обыкновенная свекловичная блоха, бересклетовая тля.

Обыкновенная или гречишная блоха (Chaetocnema concinna Marsch) относится к отряду Coleoptera сем. Chrysomelidae). Это мелкие жуки длиной 1,3–2,3 мм, черного цвета с зеленоватым или бронзовым оттенком. Усики – 11-ти члениковые. Задние ноги прыгательные, задние и средние голени у вершины с глубокой выямкой, усаженной щетинками. На переднеспинке у заднего края 2 косых вдавления и ряд крупных точек. Яйца блошек светло-желтые, вытянуто-овальные, длиной 0,6–0,7 мм. Личинки белые, длиной 1,5–2,2 мм, имеют 3 пары грудных ног, а на последнем членике брюшка 2 загнутых к верху шипика; эти шипики, а также голова и ноги буро-желтого цвета.

Биология гречишной блохи. Зимуют жуки под растительными остатками на обочинах полей, в лесополосах, в небольших канавках, в кустарниках и др.
Весной при теипературе 6–9°С жуки пробуждаются и приступают к дополнительному питанию. С мест зимовки они постепенно переселяются на многолетние гречишные растения (Rumex convertus Vild., P. convolvulus L. и марьевые (Chenopodyum album L.).
При температуре воздуха около 19–20°С жуки начинают спариваться. Самки откладывают яйца в почву на глубину 3–5 см по 4–6 штук., всего 15–40яиц. Гречишная блоха откладывает яйца у корней гречишных растений. Личинки появляются из яиц через 11–14 дней. Питаются они на корнях и примерно через месяц окукливаются в почве на глубине до 15 см. Жуки нового поколения в нашей зоне появляются в начале августа, питаются на сорняках и свекле, а в середине сентября уходят на зимовку.

Особенности повреждения. Вредят жуки и личинки. Жуки выгрызают на семядольных листочках верхний эпидермис и паренхиму. Нижний эпидермис остается целым. При дальнейшем росте листа эпидермис в местах повреждения выпадает и пятна превращаются в дырки. Жуки повреждают и подсемядольное колено, вследствии чего стебель ломается и растение гибнет, посевы изреживаются.
Повреждения всходов гречихи блохой особенно опасны в сухую, жаркую погоду. При таких условиях жуки повреждают точку роста молодых растений, что также приводит к их гибели и изреживанию всходов.
Нашими исследованиями установлено, что восприимчивость всходов гречихи к обыкновенной гречишной блошке зависит от срока посева. Ранние (апрель – первая декада мая) посевы более уязвимы к повреждению по сравнению с поздними.
В процессе исследований мы обратили внимание на то, что отдельные виды гречихи, мутанты и селекционные номера менее поражаются, на других же отмечено более сильное повреждение. Это послужило основой для составления шкалы учета степени повреждения семядольных листьев (рис. I). Вся шкала четырех бальная:
0 – повреждений не отмечено;
1 – повреждения занимают не более 10 % поверхности семядольных листьев;
2 – повреждения охватывают от II до 35 % листовой поверхности;
3 – повреждением охвачена вся листовая пластинка (более 50 % поверхности).
Эту шкалу мы использовали для изучения устойчивости селекционного материала гречихи.

Устойчивость селекционного материала
Исследования показали, что среди районированных и перспективных сортов (Виктория, Аврора, Орбита, Подолянка, Астория, Глория, Казанская, Смолевчанка, Селена, Радеховская улучшенная, Лада, Галея, Диадема, Энеида, Искра, Большевик-4, Идель, Богатырь, Калининская, Красностреле̌цкая, Пожнивная, Геркулес, Скороспелая-81, Поукосная, Зарница / высокоустойчивых к гречишной блохе не обнаружено.

Среди образцов гречихи обыкновенной Fagopyrum esculentum среднеустройчивыми были карликовые мутанты Малыш и Надежда.
При изучении гомостильных и гетеростильных растений гречихи существенных различий в степени устойчивости не выявлено. Наиболее устойчивыми (бал-I) к гречишной блохе в естественных полевых условиях были дикие сородичи гречихи обыкновенной: Fagopyrum cymosum Meissn, Fagopyrum giganteum Krot., Fagopyrum tataricum ssp. himalaicum Krot, Polygonum sachalinense, P. weurichi, P. nitens.
Эти виды представляют ценность как генетические источники в селекции гречихи на устойчивость к обыкновенной гречишной бдохе (Chaetocnema concinna Marsh) и их необходимо привлекать в скрещивания при отдаленной гибридизации.

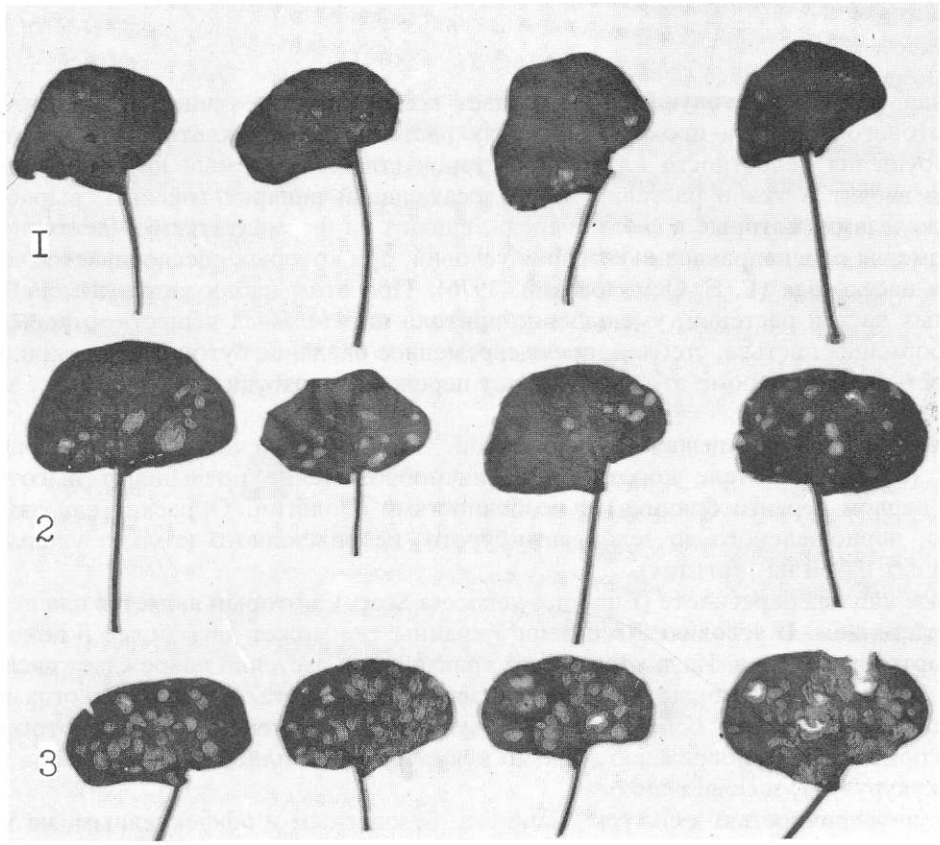


Fig. 1. Scale for calculation of the degree of injury.

Факторы устойчивости.

Отмеченные различия в степени повреждаемости различных образцов гречихи были предпосылкой к изучению причины устойчивости. Факторами устойчивости могут быть морфологические признаки (наличие кутикулы, восковой налет на листьях, опушенность растений). Просмотр и анализ этих признаков в устойчивых и восприимчивых образцов гречихи не дали существенных различий. Различия были только в размерах листовой пластинки. В тетраплоидных формах листовая пластинка была в 1,5 раза крупнее в сравнении с их диплоидными аналогами.

Обыкновенная (гречишная) блоха питается растительной тканью и устойчивость отдельных видов гречихи по-видимому обусловлена пищевым предпочтением того или иного растения. Биохимический анализ зеленых листьев гречихи показал различное содержание жизненноважных веществ.

Содержание каротина (провитамина – А) было не одинаково. Для восприимчивых образцов гречихи обыкновенной Fagopyrum esculentum в семядольных листьях содержание каротина составляло 83 мг на 100 г сухого вещества, в настоящих же листьях оно равнялось 77 мг, в то время, как в устойчивого вида Fagopyrum cymosum оно не превышало 39 мг.

Витамин »А« влияет на рост и развитие насекомого. И его высокое содержание по-видимому стимулирует насекомых к лучшему питанию.

Существенным критерием, имеющим значение в питании насекомых – листоедов, имеет нежность тканей, содержание воды, минеральных веществ и др. Грубые и жесткие растительные ткани очень редко посещают вредители. Главным компонентом, определяющим эластичность и упругость растительных тканей, является содержание клетчатки.

Анализ показал, что в семядольных листьях, которые преимущественно повреждает гречишная блоха, содержание клетчатки очень низкое (19 %), в листьях первого и второго яруса обыкновенной гречихи – 32 %, а в устойчивого вида гречихи полузонтичной её содержится – 48 %. Это дает основание предполагать, что устойчивость к обыкновенной блохе согласуется с уровнем содержания витамина »А« и клетчатки в листьях гречихи.

Бересклетовая тля Aphis evonymi L. повреждает все органы гречишного растения. Колющие щетинки ротового аппарата проходят в тканях растения по межклеточному пространству не вызывая нарушения целостности клеточных стенок (так называемый интроцеллюларный тип укола). Они вводят в ткани растений через сосательный аппарат токсины, вырабатываемые слюнными железами, которые в свою очередь, влияют на ферментативную деятелность самого растения, изменяя её в направлении создания условий, при которых обеспечивается возможность питания для насекомых (Г. Е. Осмоловский, 1976). При этом наблюдается изменение окраски поврежденных частей растений, уменьшение притока питательных веществ к формирующимся плодам, деформация листьев, стеблей, преждевременное опадание бутонов и цветков, щуплость и недоразвитость плодов. Кроме этого тля может переносить возбудителей вирусных заболеваний гречихи.

Бересклетовая тля очень близка к свекловичной. Отличается некоторыми морфологическими признаками (волоски на теле короткие, щетинкообразные не превышают высоту краевого бугорка на первом тергите брюшка) и особенностями экологии. Окраска тела изменчива, от чернобурого, черно-зеленого до зеленовато-бурого, не опыленного (только у нимф два ряда белоопыленных пятен на тергитах).

Зимует в фазе яйца на бересклете (Eunomus verricosa Scop.), который является для неё основным кормовым растением. В условиях Лесостепи Украины тля может дать более 5 поколений, размножаясь партогенетически. Из дикорастущих травянистых растений повреждает паслен черный, будяк, осот, щавель, на которые мигрирует в мае с бересклета. Однако, никогда не заселяет лебеду, марь белую, свеклу, бобы и мак. Из культурных растений летние партогенетические поколения кроме гречихи повреждают только кукурузу и подсолнечник, в то время как свекловичная тля кукурузу не заселяет никогда.

В связаи со специфичностью культуры, наиболее безопасным и эффективным, на наш взгляд, является выведение устойчивых сортов гречихи. Наши исследования направленные на поиск устойчивых форм к бересклетовой тле показали, что гомостильная форма Х. Маршалла (с короткопестичным типом цветка), мутанты салатной формы, некоторые карликовые и черноплодные формы характеризовались повышенной восприимчивостью к бересклетовой тле.

Из проанализированных 3000 образцов только дикие сородичи гречики F. cymosum, F. tataricum ssp. himalaicum, F. giganteum Krot., оказались устойчивыми к даному вредителю.

Признаки устойчивости гречихи к тлям. Выявленные различия в устройчивости были предпосылкой к изучению морфологических признаков, определяющих численность насекомых на растениях. Анализируя под микроскопом черешки листьев и цветоносов восприимчивых и устойчивых форм гречихи, мы обнаружили большой полиморфизм по наличию волосков и плотности опушения. У сильно восприимчивых образцов гречихи на стеблях, черешках листьев и цветоносах волоски отсутствовали. Более устойчивые формы имели волоски. Одноклеточные гибкие волоски размещаются по желобку и выступам черешка. С противоположной стороны черешка опушение или отсутствует или оно очень редкое. Инная картина наблюдается в устойчивых форм. Их черешки листьев и соцветий по всей окружности имели густое опушение. Различия в морфологических признаках отмечены и в строении листовой пластинки, жилкования и др. (рис. 2).

У воспримчивых форм край листовой пластинки гладкий, в то время как у более устойчивых – по краю листа наблюдается зазубренность. Это сидячие железки. Для этои группы сортов и образцов характерные маленькие и по-видимому, физиологически неполноценные одноклеточные железки. В группе иммунных форм гречихи эти железки преимущественно многоклеточные. Они состоят из ножки, погруженной в эпидермис и головки, выступающей над его поверхностью. Из кончиков волосков, железы выделяют эксудат (рис. 3).

При изучении морфологии жилок на листовой пластинке мы увидели аналогичную картину. В устойчивого вида как главная (центральная), так и все другие инервируемые от неё жилки имели опушенность. У восприимчивых – опушенность отсутствовала (рис. 4).

Отмеченные различия в опушенности привели к дальнейшему изучению поведения тлей на устойчивых и восприимчивых формах. Наблюдения за поведением подопытных насекомых показали, что на восприимчивых образцах тли интенсивно размножались, а на устойчивых (имеющих опушение) вели себя обеспокоено и с трудом передвигались (рис. 5).

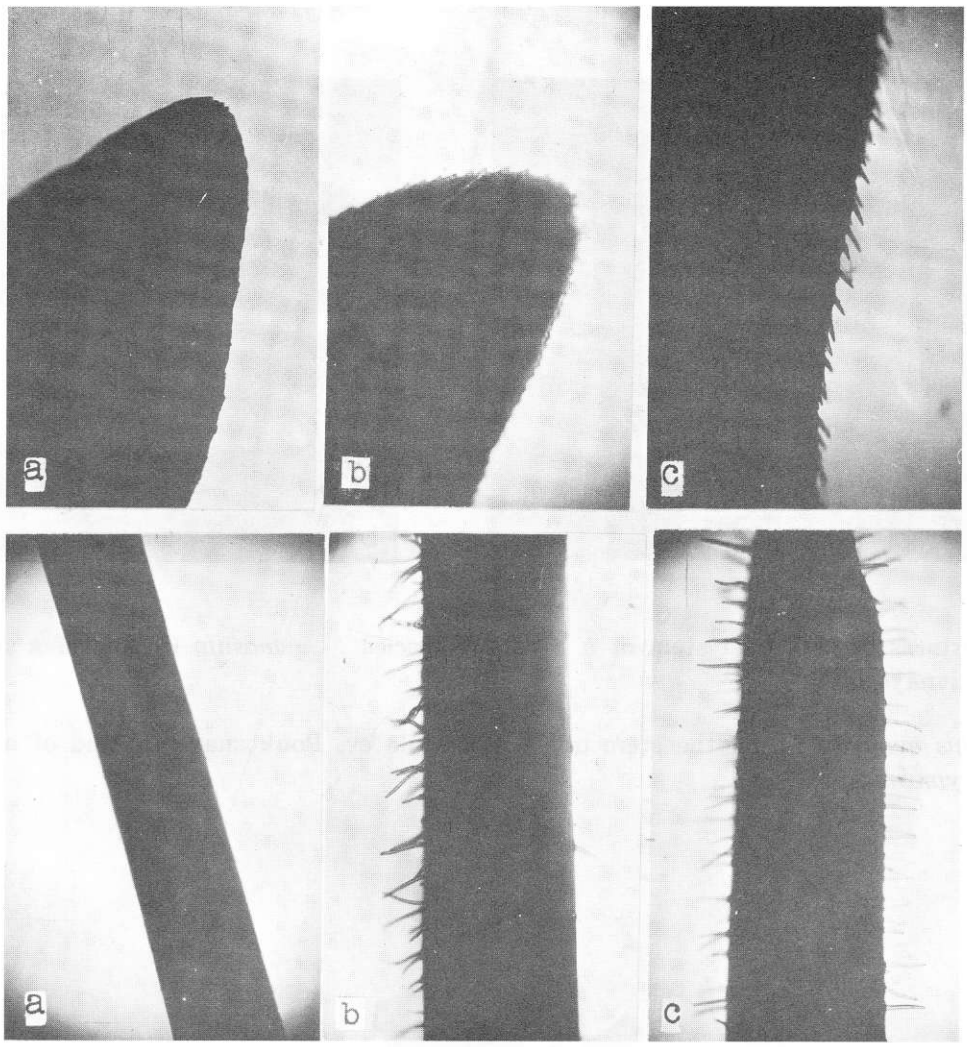


Fig. 2. Hairiness of leaves and stem of a susceptible variety Poukosnaya (a), of a green-flower form (b) and of resistant species *F. cymosum* (c).

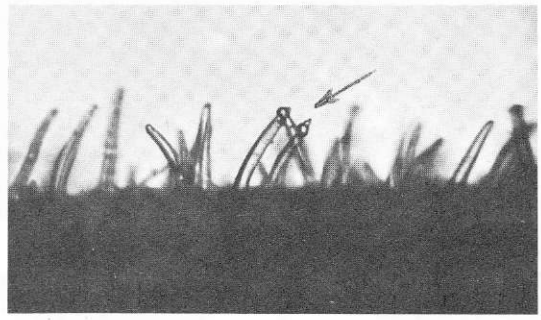
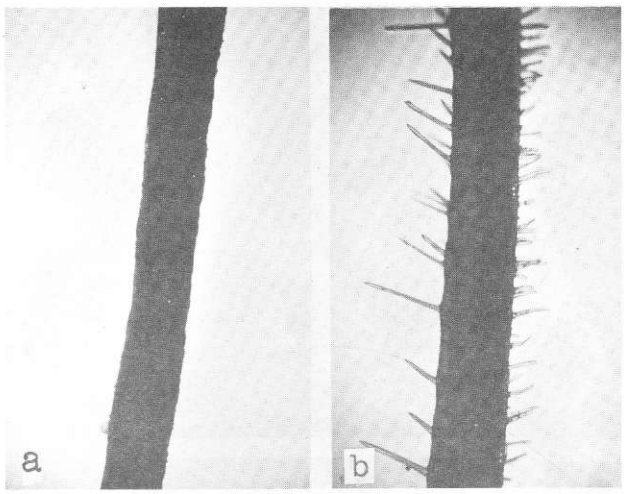


Fig. 3. Drops of exudation are shown by an arrow.



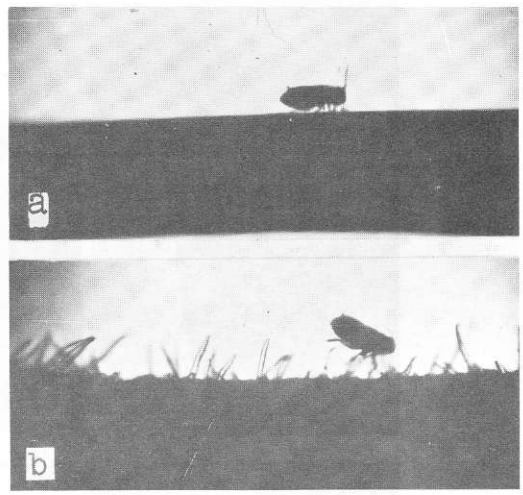


Fig. 4. Hairiness of the main stem of a resistant species *F. cymosum* (b) and of a susceptible one - Pozhnivnaya (a).

Fig. 5. *Aphis evonymi* L., on the stem of a susceptible cv. Poukosnaya (a) and of a resistant species *F. cymosum*.

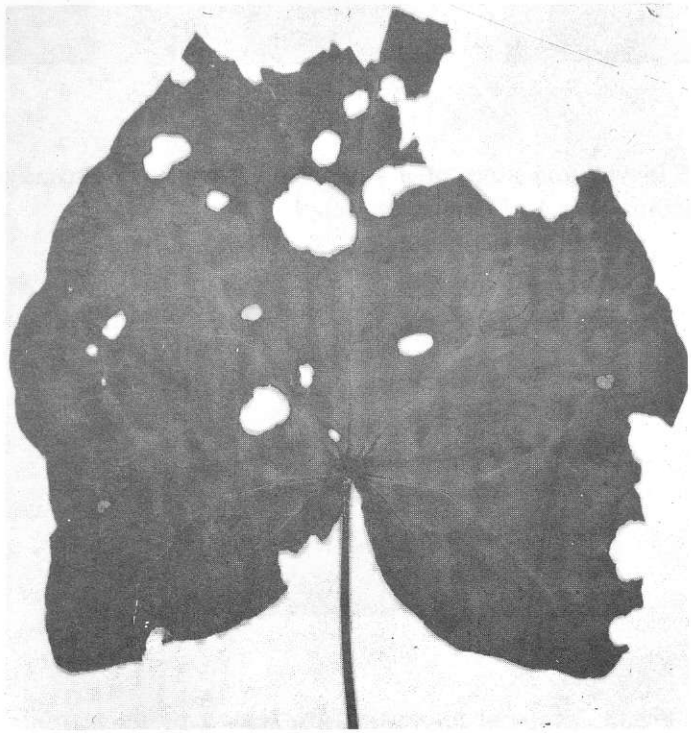


Fig. 6. Caterpillar damage to a buckwheat leaf by *Autographa gamma* L.

Стилетные концы ротового аппарата тлей из-за помех длинных волосков в эпидермисе, реже проникают в ткани устойчивых растений, что приводит их к гибели от истощения. Они погибали также быстро, как и тли не получившие пищи.

Таким образом, выявленные признаки опушенности органов растений гречихи в устойчивых форм – являются маркерными при селекции на иммунитет к тлям и одновременно к вирусам.

Из многоядных вредителей в последние годы на гречихе наиболее распространенными являются повреждения гусеницами совки-гамма Autographa gamma L, медведки Gryllotalpa gryllotalpa L., озимой совки Agrotis segetum L. и др.

Нами отмечено, что гусеницы совки-гамма, которые в ночное время повреждают настоящие листья, выедают в них округлые дырочки (рис. 6).

Устойчивых форм гречихи к этому вредителю не выявлено.

Гречиха прекрасный медонос. Её посевы привлекают множество опылителей и других полезных насекомых-энтомофагов, которые по-видимому регулируют численность вредных видов.

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DEFENSE MECHANISM OF BUCKWHEAT AGAINST SOME PESTS

Abstract

The work presents the biology of the most widespread buckwheat pests in USSR: *Chaetocnema concinna* and *Aphis evonymi*. Factors influencing the resistance of buckwheat species *Fagopyrum esculentum* and *Fagopyrum cymosum* to the mentioned pests were studied. The hairiness of the stems seems to be an important factor of resistance.

OBRAMBNI MEHANIZMI AJDE PRED NEKATERIMI ŠKODLJIVCI

Izvleček

V delu je predstavljena biologija najbolj razširjenih škodljivcev ajde v Sovjetski zvezi. Ta škodljivca sta *Chaetocnema concinna*, ki spada v družino lepenjcev in v red hroščev, ter *Aphis evonymi*, ki spada v družino listnih uši ter red enakokrilcev. Delo vsebuje opis biologije obeh vrst, poškodbe, ki jih povzročata škodljivca, ter obrambni mehanizmi dveh vrst ajde (*Fagopyrum esculentum* in *Fagopyrum cymosum*), s katerimi so rastline zavarovane pred napadom. Izgleda, da so za obrambo pomembne morfološke in nekatere fiziološke oziroma biokemične lastnosti.

GOSPODARSKO POMEMBNE BOLEZNI AJDE V SOVJETSKI ZVEZI

Izvleček

V Sovjetski zvezi so pri ajdi gospodarsko pomembne tri glivične bolezni (*Peronospora fagopyri, Botrytis cinerea* in *Ascohyta fagopyri*), ena bakterijska bolezen (*Pseudomonas syringae*) in virusni ožig. Simptomi teh bolezni so opisani po opazovanju avtorjev.

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О БОЛЕЗНЯХ ГРЕЧИХИ

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Представлены данные о наиболее распространенных болезнях гречихи.

Длительное время считалось, что гречиха не поражается болезнями. Однако, к настоящему времени на культуре гречихе зарегистрировано более 30-ти возбудителей заболеваний. К ним относятся грибы, бактерии, вирусы. Заболевания растений проявляются в увядании, гнили растений, пятнистости, хлорозе листьев и др.

Из возбудителей **грибных заболеваний** на гречихе зарегистрированы: Peronospora fagopyri Elenev, Botrytis cinerea Fr., Ascochyta fagopyri Bres, Fusarium oxysporum (Schlecht) Snyd. et Hans., F. heterosporum Fr., Phytophthora parasitica Dast., Ph. fagopyri Takimato, Erysiphe communis (Wallr.) Crev f. fagopyri Jacz., Phyllosticta polygonorum Sacc., Ramularia fagopyri Abr. Ramularia curvula Fautr., Cercospora fagopyri Abram., Fusicladium fagopyri Oud., Cladosporium herbarum Lk., Sphacelotheca fagopyri Syd. et Butl., Whetzelinia fagopyri (Hori) M. Chochr. (syn. Sclerotinia fagopyri Hori), Hypochnus solani Pr. et Del., Puccinia fagopyri Bazel.

Бактериальные болезни: Bacterium solanacearum Smith, Pseudomonas syringae Van. Hall., Bacterium proteamaculans (Paene et Stansfield)., Xantomonas heteroceae (Wsonoh) Savulesku, Pseudomonas angulata (Fromme et Murroy) Holland.
(М. К. Хохряков и др. 1984).

Вирусные болезни: **ВТМ (вирус табачной мозаики)** – Ruga verrucosans K. Smit (1960), а также вирус курчавости герани (Ferault A.C., 1980), бацилловидный вирус (Е. С. Алексеева и др, 1988) и др. Возбудители заболеваний повреждают растения гречихи в различные периоды вегетации (табл I).

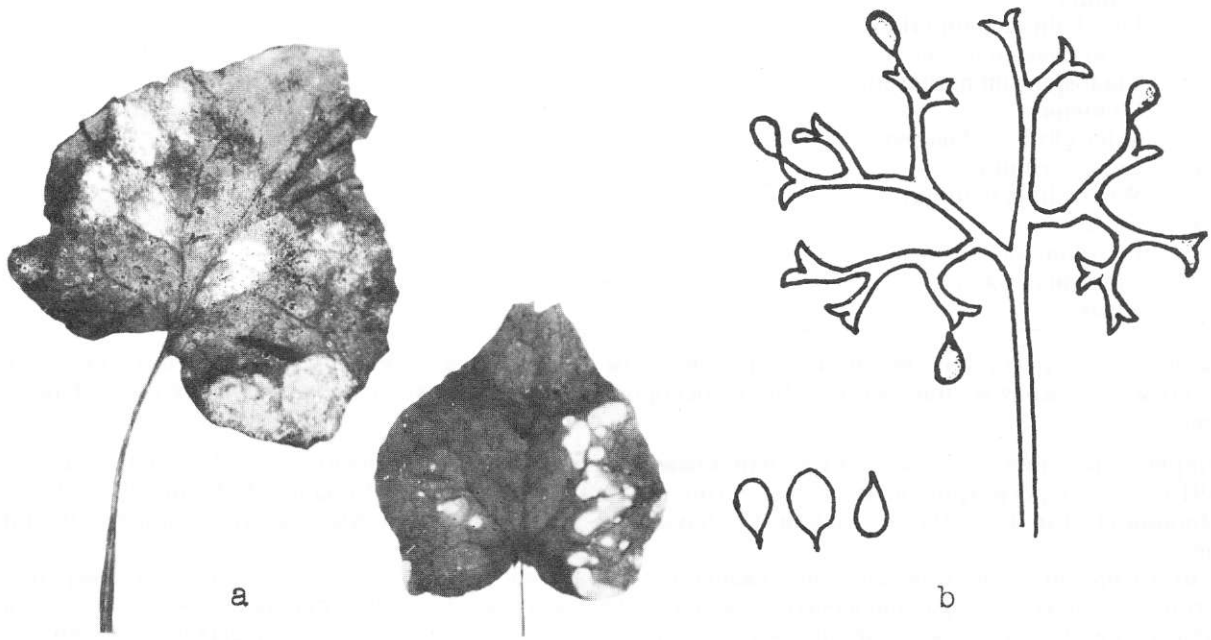

Fig. 1: *Peronospora fagopyri* Elenev on leaves (a) and spore-bearing part (b).

Таблица I.

Проявление заболеваний гречихи по фазам развития

Но. п/п	Название заболеваний	листья		Фази развития растений гречихи		
		Семядольные	Настоящие	бутонизация	цветение	плодообразование
1.	Пероноспороз Peronospora fagopyri	–	+	+	+	–
2.	Серая гниль Botrytis cinerea	+	–	–	+	+
3.	Фузариоз Fusarium heterosporum, Fusarium oxysporum	–	–	–	+	+
4.	Фитофтора Phytophthora parasitica, Ph. fagopyri	+	–	–	–	–
5.	Белая ножка Hypochnus solani	–	–	–	+	+
6.	Мучнистая роса Erysiphe communis	–	–	+	+	+
7.	Филлостиктоз Phyllosticta polygonorum	+	+	+	+	+
8.	Рамуляриоз Ramularia fagopyri, Ramularia curvula	–	–	–	+	+
9.	Церкоспороз Cercospora fagopyri	–	–	–	+	+
10.	Аскохитоз Ascochyta fagopyri	–	–	–	+	+
11.	Парша Fusicladium fagopyri	–	–	–	+	+
12.	Оливковая плесень Cladosporium herbarum	–	–	–	+	+
13.	Головня Sphacelotheca fagopyri	–	–	–	–	+
14.	Склеротиниоз Whetzelinia fagopyri	–	–	–	–	+
15.	Бактериоз Pseudomonas syringae	–	–	–	+	+
16.	Вирусный ожог Virus	–	–	+	+	+

К наиболее распространенным и вредоносным болезням в основных районах возделывания относятся ложная мучнистая роса (переноспороз), серая гниль, аскохитоз, вирусный ожог, бактериоз.

Первые сведения о поражении гречихи **ложной мучнистой** росой относятся к 1910 г. (Ducomet V., 1910), она зарегистрирована повсеместно в стране, а также в Польше (T. Siemaszko, 1929), Японии (J. Tanaka, 1934), Румынии, (T. Savulesku, 1948), Северной Америке (R. Zimmer, 1978) и др.

Симптомы болезни. Болезнь обнаруживается в фазе настоящих листьев, на которых с верхней стороны образуются расплывчатые, желтоватые пятна, а с нижней – рыхлый серо-фиолетовый налет (рис Iа). Пораженные цветки приобретают коричневую окраску, засыхают. Развитию болезни способствует повышенная влажность воздуха.

Возбудитель заболевания – низший гриб Peronospora fagopyri Elenev класса Oomycetes, порядка Peronosporales. Он образует межклеточный мицелий, поверхностное канидиальное спороношение, а в пораженных тканях ооспоры. Конидиеносцы разветвлены дихотомически, размером 380–500 х 8 – 12 мк, с концевыми прямыми веточками длиной 8–16 мк (Рис 1.в). Ооспоры шаровидные, гладкие, коричневые в диаметре 22–25 мк.

Первичное заражение происходит при помощи ооспор, а вторичное (распространение во время вегетации растений) – при помощи конидий. Инфекция переноспороза сохраняется в форме ооспор на остатках растений, а иногда и в оболочке семян.

Возбудитель переноспороза гречихи является узкоспециализированным паразитом.

Серая гниль гречихи впервые описана на гречихе Н. А. Наумовым (1937). Она поражает гречиху во всех районах возделывания гречихи и наносит значительный вред ее посевам. Больные растения отстают в росте. Потери урожая достигают 15,6 % (И. И. Иодко, 1972).

Симптомы проявления. Заболевание характеризуется образованием на листьях, стеблях и соцветиях бурых гниющих пятен, которые покрываются серой плесенью и черными пленками. Стебли во влажную (Рис 2 а, в, с) погоду ламаются, что приводит к преждевременной гибели растений.

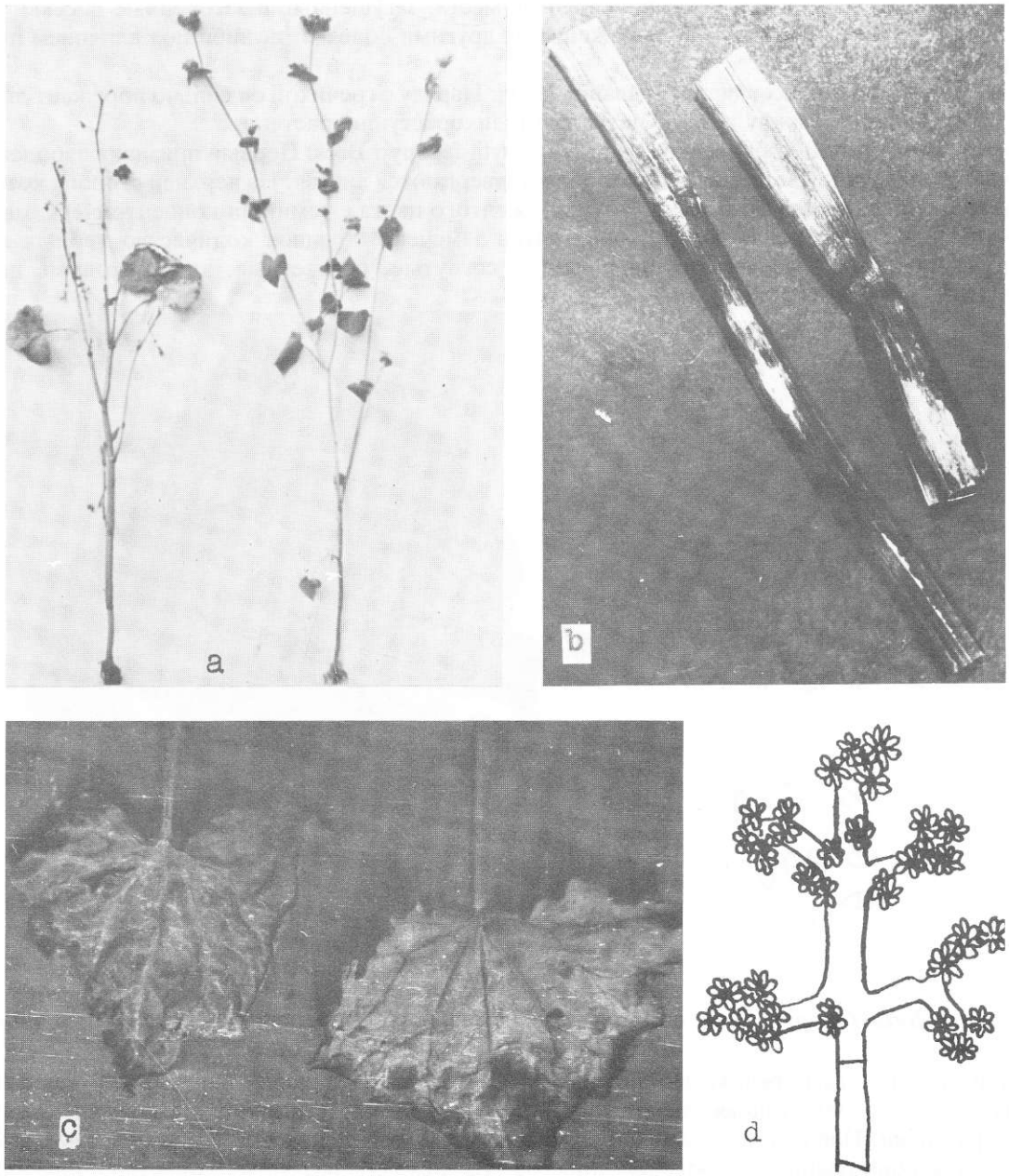

Fig. 2: Plant damaged by botrytis cinerea Pers. and a sound plant of buckwheat (a). Botrytis cinerea Pers. on stems (b), on leaves (c) and spore-bearing part (d).

При поражении всходов в нижней части стебля у корневой шейки и на подсемядольном колене образуются буроватые пятна, такие стебли утончаются и загнивают вместе с семядолями, что приводит к изреживанию посевов. При избыточных осадках инфекция распространяется и на плоды. что снижает всхожесть семян на 10–15 %.

Возбудитель заболевания – несовершенный гриб Botrytis cinerea Fr. класса Fungi imperfecti, порядка. – Hyphomycetales.

Гриб имеет серо-оливковую грибницу и образует обильное конидиальное спороношение. Конидиеносцы древовидные с перегородками, разветвленные с утолщенными окончаниями. Конидии яйцевидные или округлые, одноклеточные (10–17 х 7–10 МК), (Рис 2).

Гриб распространяется конидиями, а зимует в форме склероциев. Развитию заболевания способствуют повышенная влажность, пониженные места, загущенные и засоренные посевы, ослабление гречишных растений из- за поражения их другими болезнями, либо под влиянием низких температур и заморозков.

Botrytis cinerea – широко специализированный вид. Наряду с гречихои он сильно поражает люпин, горох, вику, капусту, свеклу и др. культурные и дикорастущие растения.

Аскохитоз. Возбудитель заболевания гриб Ascochyta fagopyri Bres. Первые признаки заболевания наблюдаются в начале цветения. Заболеванию подвергаются листья. На верхней стороне которых образуются большие округлые пятна охряно – желтого цвета с темными концентрическими кругами 4–10 мм в диаметре. В центре таких пятен замечено большое количество черных точек пикнид. В пикнидах находятся цилиндрические согнутые, бесцветные, двухклеточные пикноспоры (16–18 х 6–7 МК) (Рис 3).

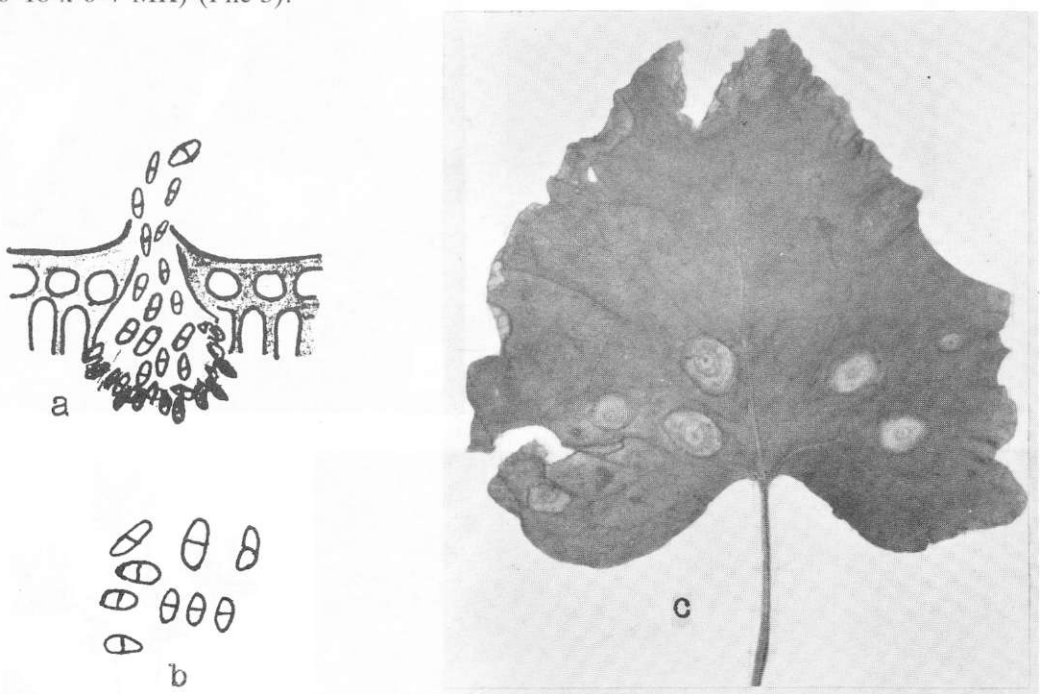

Fig. 3: *Ascochyta fagopyri* Bres. on leaves of buckwheat (c), a picnid (a), and picnospore (b).

Большой вред посевам гречихи в основных районах ее возделывания наносит **вирусный ожог.** Признаки заболевания становятся заметными в начале фазы бутонизации. Растения отстают в росте и развитии. Наблюдается сближение междоузлий, утолщение узлов. В некоторых случаях наблюдается образование 1–3 боковых побегов. Происходит засыхание соцветий, образование недоразвитых побегов и цветков, завязывание щуплых семян. Фазы бутонизации и цветения очень растянуты.

Растения остаются низкорослыми с недоразвитыми генеративными органами до конца вегетации и напоминаютт »ведьмины метлы«. Болезнь может поражать и отдельные ветки. Для молодых листьев характерным является прозрачность жилок. Старые листья внезапно покрываются некротическими пятнами, засыхают и опадают. Отчего растения кажутся как-бы обгорелыми (Рис 4 а, в, Рис 5 а).

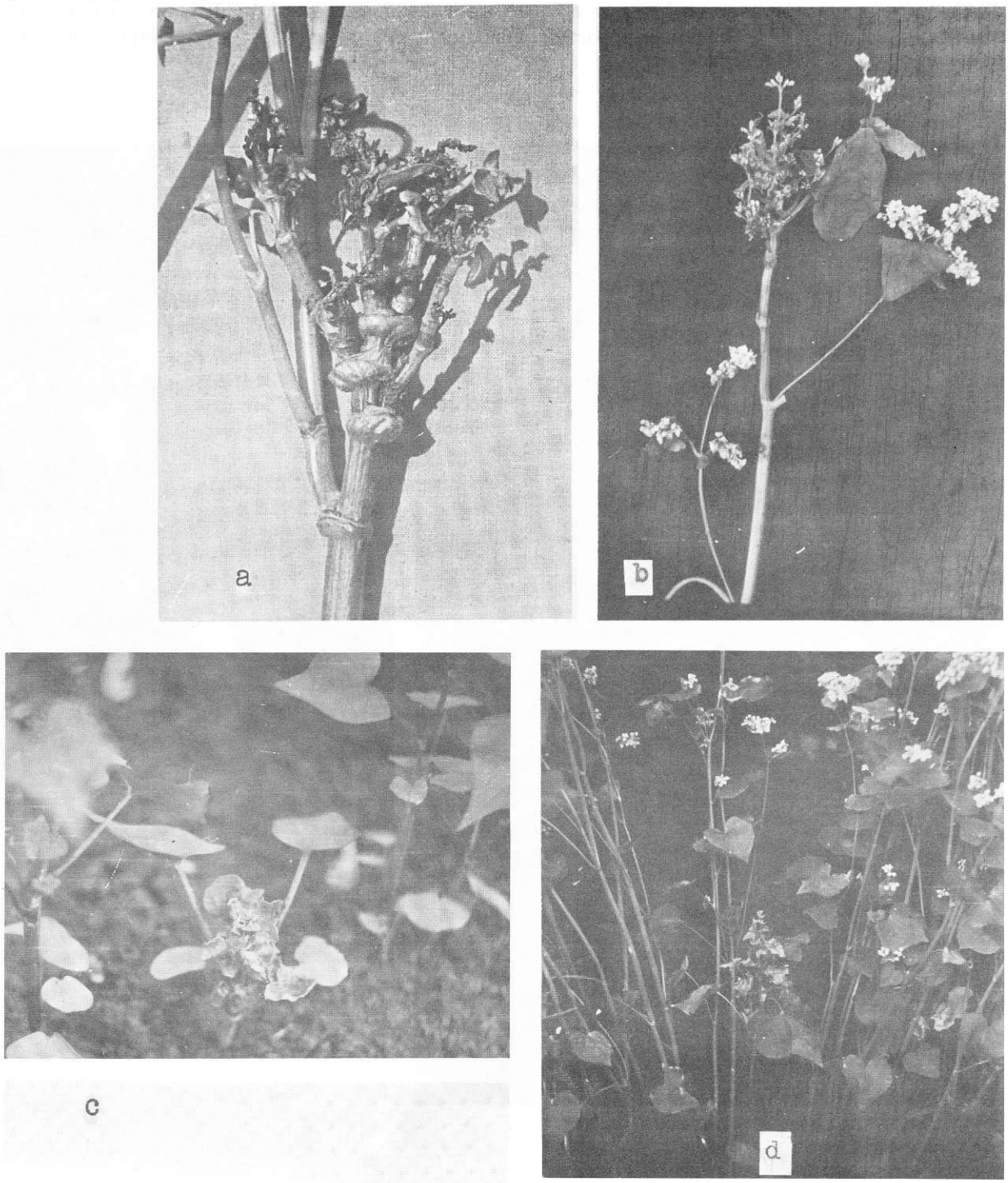

Fig. 4: Virus burn on stalk (a), on top inflorescence (b). Diseased plants in a sparse sowing (c) and in dense one (d).

Вредносность вирусного ожога прежде всего проявляется в снижении высоты растений в сравнении со здоровыми на 36–52 %. Болезнь приводит к слабой завязываемости плодов, резкому снижению продуктивности растений, которая уменьшается на 75–85 %. Такие растения формируют плоды низкого качества, не пригодные для использования в качестве семенного материала. Его возбудителем является бацилловидный вирус (Рис 5).

Большое влияние на развитие вирусного ожога гречихи оказывают сроки, способы и нормы высева. Наивысший процент больных растений наблюдается при поздних (июньский) сроках сева в разреженных посевах.

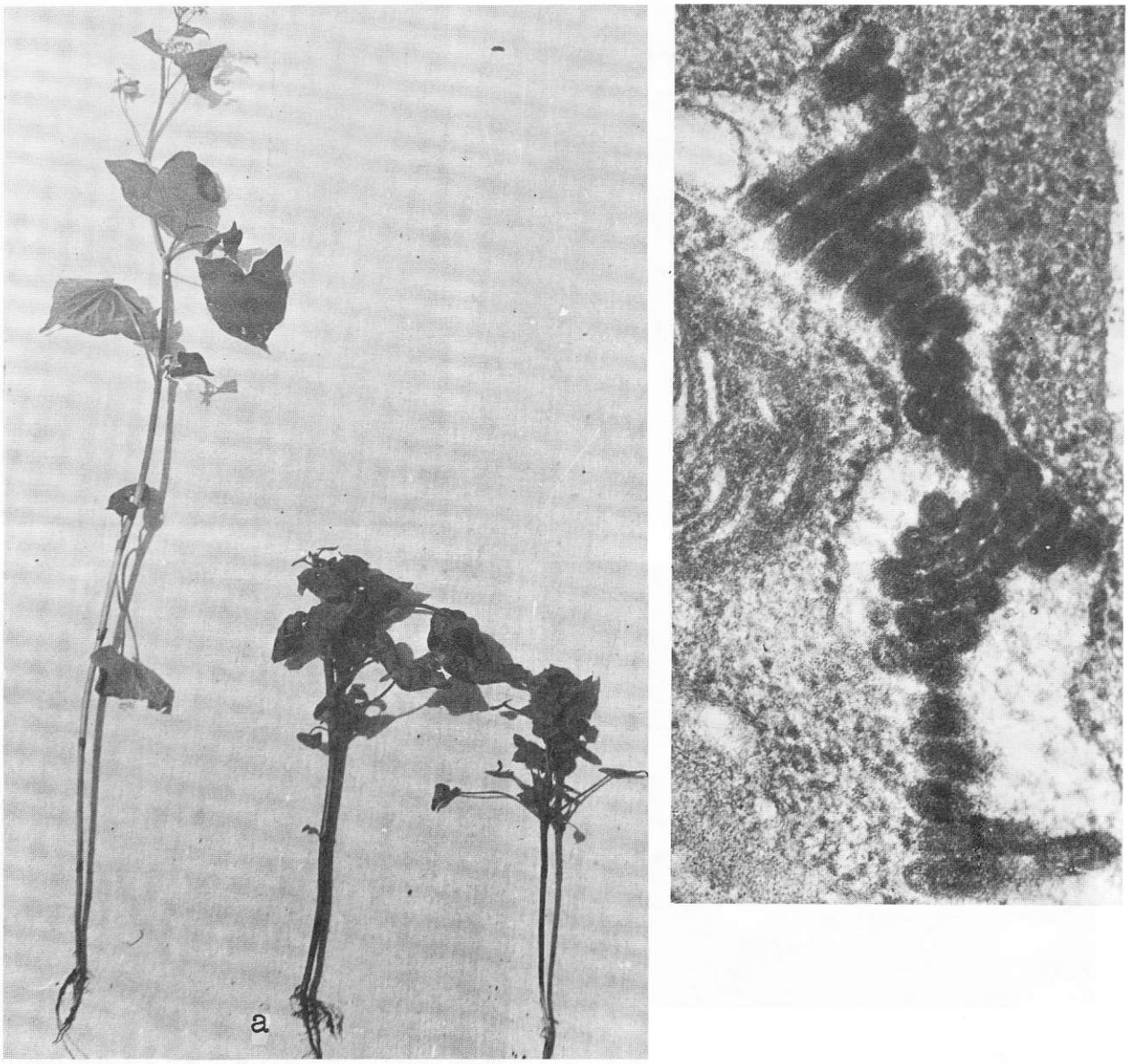

Fig. 5: Virus burn. General view of a sound and damaged plant (a). Bacillary virus (enlargement 60,000 x).

Бактериоз. Первые признаки болезни отмечаются в фазе бутонизации. На листьях образуются сначала единичные красно – бурые пятна, более или менее округлые. В центре пятен замечены блестящие пленочки, которые представляют собой эксудативный налет. С нижней стороны пятна кажутся как-бы вдавленные. По мере развития заболевания пятна охватывают всю листовую пластинку. Лист коробится, засыхает и опадает. Инфицируются также стебли, формирующиеся семена, цветки. Возбудитель заболевания бактерия Pseudomonas syringae Van. Hall. (Рис 6).
Непременным условием в повышении устойчивости гречихи к заболеваниям является создание оптимальных условий для роста и развития растений.

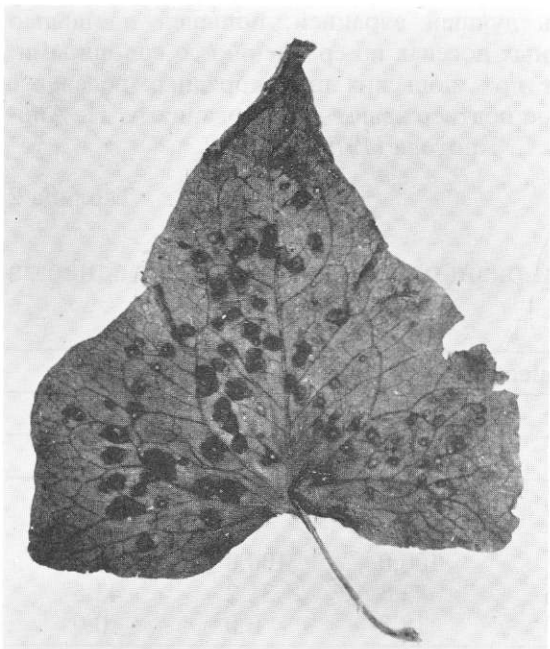

Fig. 6: Bacteriosis in buckwheat.

Известно, что высококачественные семена – залог стабильных высоких урожаев гречихи. Большое значение в повышении болезнеустойчивости гречихи имеет тщательная очистка и калибровка семян. Этот прийом дает возможность удалить щуплые, недоразвитые семена, которые чаще несут инфекцию многих возбудителей болезней. Крупные фракции семян обычно дают дружные всходы из которых развиваются здоровые продуктивные растения.
В повышении болезнеустойчивости гречихи немаловажную роль играет прогревание семян (П. В. Пак, 1973, 1980). Этот метод широко применяется в семеноводстве в борьбе с пыльной головней шпеницы, ячменя и других культур. По данным И. И. Иодко (1972) из прогретых семян (в воде при температуре 50° в течении 2 часов), поражаемость растений серой гнилью снизилась в 2,7 раза, урожай повысился на 2,1 ц/га.
Одной из причин поражаемости гречихи болезнями является размещение ее после случайных засоренных предшественников и выращивание по примитивной агротехнике.
После предшественников, которые оставляют почву засоренной, гречиха резко снижает урожай, хотя в первой половине своего развития она »подавляет« сорняки. Однако, в период массового цветения, а также побурения плодов рост растений приостанавливается, а сорняки интенсивно растут, используя запасы влаги и элементы питания из почвы, опережая в росте растения гречихи и затеняя их. Все это в большой степени ухудшает сопротивляемость гречихи к комплексу заболеваний. По данным А. Н. Анохина, И. И. Иодко (1973) при посеве гречихи сплошным способом в третей декаде мая ее растения (без прополки сорняков) были поражены серой гнилью и вирусным ожогом на 15,9 %, а с прополкой на 10,8 %. При широкорядном способе посева число сорняков было намного ниже, и поражаемость соответственно составляла 7,8 % и 6,3 %. Некоторые сорняки являются резерваторами возбудителя вирусного ожога гречихи, например P. convolvulus.

Большую роль в поражаемости растений и интенсивности развития болезней играют экологические условия, особенности взаимоотношений рстений, что в первую очередь обуславливается сроками, способами и нормами высева, и считается одним из самых значительных резервов повышения урожая.

В зависимости от сроков посева восприимчивость гречихи к вирусному ожогу была не одинаковой (табл. 2). Растения ранних сроков сева (апрель, май) незначительно поражались вирусным ожогом по сравнению с поздними. Начиная с последней декады мая и до конца июля поражаемость возростала. Причем, у гречихи татарской она достигала 100 %.

На восприимчивость гречихи к вирусному ожогу большое влияние оказывает и густота стояния. При сплошных посевах поражаемость гречихи вирусным ожогом была в четыре раза большей в сравнении с широкорядным, что можно объяснить лучшей аэрацией, большей площадью питания, хорошим развитием растений на широкорядных посевах по сравнению со сплошными. Наибольшего же развития вирусный ожог достигает при июльских широкорядных посевах с пониженными нормами высева (Рис 4 с, а). Эти данные подтверждают исследования А. Н. Анохина, И. И. Иодко (1973), С. Ф. Сидоровой (1965), А. С. Кротова (1979) и др.

Таблица 2

Поражаемость гречихи вирусным ожогом при разных сроках сева, % (среднее за 3 года).

Месяцы	F. esculentum			F. tataricum		
	Декады					
	I	II	III	I	II	III
Апрель	–	–	0,8	–	–	2,5
Май	0,0	0,7	0,7	3,4	1,4	35,9
Июнь	18,8	15,9	22,4	100,0	100,0	100,0
Июль	20,4	19,4	17,6	43,5	31,2	43,5
Август	6,8	0,0	0,0	18,8	0,0	0,0

А. Н. Анохин и И. И. Иодко на основании многолетних исследований предполагают, что степень заболеваемости вирусными болезнями гречихи связана с периодом максимальной солнечной активности в одинадцатилетнем цикле. Эту гипотезу (о влиянии солнечной активности в 11-летнем цикле) на распространение вирусных заболеваний на других культурах выдвигают А. Л. Чежевский и Ю. Г. Шипина (1969).

Большое влияние оказывают способы посева и нормы высева и на развитие такой болезни гречихи как серая гниль.

Исследования И. И. Иодко (1972) показывают, что поражаемость серой гнилью в широкорядных посевах была в 1,5–2,0 раза меньше, чем в сплошных.

Следует также отметить и тот факт, что с повышением нормы высева поражаемость серой гнилью увеличивается: при сплошном посеве с НВ = 3,5 млн. зерен на га поражаемость всходов составляла 27,7 %, при норме 4,5 млн/га она возросла до 40,9 %. Это связано с особенностями микроклимата. Установлено, что B. cinerea Pers. хорошо развивается при повышенной влажности воздуха и недостатке света. В широкорядных посевах создаются лучшие условия для обогрева и освещенности растений, быстрой испараемости влаги с их поверхности, что снижает интенсивность развития болезней.

Возможность использования удобрений с целью повышения устойчивости растений к вредным патогенам была доказана Т. Д. Страховым (1956). Он установил, что регулируя режим питания растений, можно изменить устойчивость их к тому или иному заболеванию.

Вопрос роли удобрений в болезнеустройчивости гречихи изучен мало. Наши опыты проведенные в бессменной культуре по изучению влияния разных доз минеральных удобрений на устойчивость растений гречихи к серой гнили показали, что поражаемость этим возбудителем согласуется с дозой и соотношением элементов питания (табл 3).

Таблица 3

Поражаемость гречихи сорта Виктория возбудителем серой гнили в бессменной культуре на разных фонах минерального питания.

Варианты	Поражаем. растений, %		
	1980	1981	Среднее
Контроль (без удобрений	7,0	6,6	6,8
N $60^{P}60^{K}$ 60	24,5	15,5	20,0
N $120^{P}60^{K}$ 60	54,8	27,8	41,3
N $60^{P}60^{K}$ 60	32,7	21,5	27,1
N $60^{P}60^{K}120$	28,1	32,1	30,4

Наивысшая поражаемость серой гнилью наблюдалась в варианте где испытывалась двойная доза азота. Двойная доза калийных удобрений увеличила заболеваемость на 10,4 % в сравнении с N $40^{P}60^{K}60$, а фосфорных занимает промежуточное положение.

С. Ф. Сидорова (1965) установила, что фосфорные удобрения дают высокий процент поражения растений возбудителем серои гнили.

Дополнительное внесение фосфорных удобрений в комбинации с калийными почти не изменяют картины. Но когда к этим удобрениям добавились азотные, процент поражения растений снизился в 1,5 раза.

По исследованиям И. И. Иодко (1972) хорошие результаты дает обработка семян гречихи раствором молибденовокислого амония, что способствует повышению устойчивости гречихи к серой гнили более чем в 2 раза. А при внесении минеральных удобрений N $40^{P}40^{K}40$ по фону доломитовой муки поражаемость возбудителем серой гнили составила 3,5 % против 6,5 % на контроле.

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ECONOMICALLY IMPORTANT BUCKWHEAT DISEASES IN USSR

Abstract

Diseases of some importance in buckwheat in the Soviet Union include three fungal diseases (*Peronospora fagopyri, Botrytis cinerea* and *Ascohyta fagopyri*), one bacterial disease (*Pseudomonas syringae*) and Virus burn. Symptoms of these diseases are described based on our own observations.

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Влияние Мивала и Крезацина на морфологические признаки, урожай соломы и зерна, а также содержание белка и его аминокислотной состав в зерне гречихи (Fagopyrum esculentum Moench).

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Введение

Хотя, гречиха в мировом земледелии не имеет широкого распространиения, площади посева её занимают большой ареал в Польше и СССР (7). В связи с высоким содержанием в плодах ценных питательных веществ (высоко качественни белки (2,8) гречиха является предметом исследований многих учёных (3,4 5,8). Возможность широкого использования зерна, соломы, а также зеленых растений в питани людей и животных (1,6) создает стимул для проведения исследований над этой культурой. Одной из возможностей увеличения урожая гречихи и улучшения его качества является поиск и применение соответствующих регуляторов роста растений. Об этом наглядно свидетельствуют работы, появляющиеся в последнее время (9, 10).

Материал и методы исследований

В рамках сотудничества в Комплексной программе научно – технического развития стран-членов СЭВ по теме 5.1.2.3: »Разработка технологии применений регуляторов роста растений« в с/х академии в Люблине провели изучение влияния на гречиху двух новых синтетических регуляторов роста – Мивала и Крезацыны, созданных в СССР. Активным веществом препарата Мивал является I-хлорметилсилатран, Крезацин же – это триэтанолоаминовая соль крезоксиуксусной кислоты. Семена гречихи намачивались в водных растворах исследуемых веществ в 3 концентрациях. Схема опыта следующия:

Вариант Combination	Регулятор роста Growth regulator	Концентрация (мг/дм^3) Concentration (mg/dm^3)
1	Сухие семена No soaking	контроль control
2	Намоченные в воде Soaking in water	контроль control
3	Мивал Mival	200
4	Мивал Mival	500
5	Мивал Mival	1000
6	Крезацын Krezacyne	10
7	Крезацын Krezacyne	25
8	Крезацын Krezacyne	50

Мелкоделяночный опыт был проведен в учхозе фелин. До посева на участке внесли минеральные удобрения в количестве 60 кг N, 80 кг $P_2 0_5$ и 10 кг $K_2 0$/га. В период вегетации наблюдали за фазами роста и развития растений гречихи. С каждой делянки на 10 зрелых растениях определили длину надземной части растений, число междоузлий, веток, соцветий, а также число и массу сформированных семян. Учитывали урожай зерна и соломы, а также массу 1000 зерен. В зерне определили общее содержание белка на приборе Кьель-фосс. Провели, также, анализ аминокислотного состава белка методом колонковой ионообменной хроматографии в анализаторе типа АА 881. Результаты биометрического анализа и урожайность обработали статистически, подсчитивая доверительные полупределы Такея.

Обсуждение результатов

Обработка семян гречихи препаратами Мивал и Крезацын не оказала большего влияния на фазы развития растений. На всех вариантах опыта сроки всходов, формирования первой пары настоящих листьев, образование боковых побегов, цветения и созревания были сближены.

Помимо отсуствия в биометрии существенных статистически различий удалось заметит некоторые изменения под влиянием примененных регуляторов (табл. 1). Мивал в высоких концентрациях (500 и 1000 мг/л) бызвал снижение высоты растений гречихи, зато довольно отчетливо способствовал увеличению числа веток. Очевидно, в этом проявился ретарданционный эффект. Оба регуляторы роста оказали отрицательное влияние на число и массу сформированных семян, в сравнению с контролом 2 (семена замоченные в чистой воде). Снижение массы и числа семян было найбольшим при высоких концентрациях препаратов. Урожай зерна и соломы и масса 1000 зерен отличались друг от друга статистически несущественно (табл. 2). Можно заметит полезное влияние на урожай зерна препарата Крезацына, примененного в ниской концентрации (вариант 6). Во всех вариантах опыта отмечено снижение урожая соломы по сравнению с контролем 2. Довольно отчетливое понижение массы 1000 зерен вызвали оба препарата, примененные в высоких концентраниях.

Содержание общего белка в зерне гречихи во всех вариантах опыта колебалось в пределах 12.75–13.35% (табл. 3), причем меньше всего было его в зерне на контроле 1 (сухие семена). Несколько больше этого ценного вещества было в плодах растений, выращенных под влиянием Мивала и Крезацына в высоких концентрациях – варианты 4,5, а также 7 и 8.

Регуляторя роста модифицировали содержание аминокислот в белке семян гречихи, хотя это не были крупные количественные изменения (табл. 3). Оба препарата в высоких концентрациях понизили содержание лизина и агринина, а также глютаминивой кислоты, что следует счестить неблагоприятным действием. Зато повышение содержания таких экзогенных аминокислот, как лейцин, изолейцин, цистеин и метионин, отмеченное в белке зерна гречихи под воздействием Милава и Крезацына в высоких концентрациях, является, несомненно, положительным фактором. В общем изменение концентрации аминокислот носит положителный характер, на что указывают величины показателей ИНАК для белка зерен гречихи с вариантов 4,5,7 и 8 по сравнению с контролем – 2.

Выводы

На основе полученных результатов можно сделать следующие выводы.

1) препараты Мивал и Крезацин, примененные для замачивания семян гречихи, не вляют существенным образом на фазы развития растения,
2) морфологические признаки спелых растений гречихи изменялись незначительно под влиянием примененных регуляторов,
3) исследуемые препараты не показали полезного влияния на размеры урожаев зерна и соломы, а также МТЗ,
4) довольно высокое содержание белка в зерне гречихи модифицировалось в незначительной степени Мивалом и Крезацыном,
5) суммарные изменеия содержания аминокислот в белке, вызванные регуляторамь роста, носили положительный характер, содержание многих аминокислот (изолейцина, лейцина, валина, цистеина) увеличилось,
6) на основе полученных результатов нельзя ещё сделать однозначных выводов относительно перспектив применения препаратов Мивал и Крезацын для замачивания семян гречихи.

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ТАБ. 1: Результаты биометрического анализа растений гречихи.
Results of biometric measurements of buckwheat plants.

Номер комбинации	Высота растений	Число междоузлий	Число разветвлений	Число соцвети	Число семян сформированных	Масса семян сформированных
Combination	Plants heigh (cm)	Number of internodes	Number of branchings	Number of racemes	Number of seeds	Mass of seeds (g)
1	74,1	7,8	1,6	7,2	48,6	1,36
2	73,0	7,4	1,5	7,0	60,2	1,82
3	74,2	8,0	1,9	7,9	51,9	1,54
4	72,5	7,4	2,0	6,9	52,7	1,72
5	70,2	7,3	1,9	7,7	46,2	1,50
6	75,1	8,0	1,6	7,8	56,1	1,59
7	72,5	8,1	1,4	6,8	58,4	1,69
8	75,7	7,9	1,8	7,1	54,7	1,43
$HCP_{0,05}$ LSD	–	–	–	–	–	–

Аннотация

Исследовано воздействие двух новых регуляторов роста – Мивала и Крезацина на гречиху. Установлено, что препараты не оказывают влияния на прохождение фаз равития растений гречихи. Не замечено также статистических различий в биометрии. Оба регуляторы снизили урожай соломы, а Крезацин в ниской концентрации несколько повысил урожай зерна.
Мивал и Крезацина не оказали существенного влияния на содержание белка в зерне гречихи, однако, изменили количественные соотношения экзогенных аминокислот, повышая содержание изолейцина, лейцина, валина, метионина, цистеина и понижая – лизина и аргинина.

ТАБ. 2: Влияние регуляторов роста на урожай зерна и соломы (т/га) и массу тысячи зерн – МТЗ (г)
Effect of growth regulators on seed and straw yield (t/ha) and on mass of thousand seeds (MTS in g).

Комбинация Combination	Зерно Grain	Солома Straw	МТЗ MTS
1	1,62	4,70	27,7
2	1,80	5,97	27,9
3	1,86	5,82	28,2
4	1,79	5,41	28,0
5	1,78	5,59	26,2
6	1,94	5,49	27,4
7	1,73	5,52	26,9
8	1,70	5,37	26,0
$НСР_{0,05}$ LSD	—	—	—

ТАБ. 3: Содержание общего белка (%Н ·6,25) и его аминокислотной состав в зерне гречихи (г/100 г белка).
Content of crude protein (%N x 6.25) and aminoacid composition of buckwheat grain (g/100 g protein).

Комбинация		1	2	3	4	5	7	8
Crude protein Общий белок		12,75	13,15	13,05	13,35	13,30	13,25	13,25
Лизин	Lys	6,27	6,21	6,32	5,55	5,10	5,57	5,40
Аргичин	Arg	6,11	5,50	5,52	5,09	5,20	5,35	5,18
Изолейцин	Ileu	3,21	2,89	2,90	3,37	3,68	3,72	3,51
Лейцин	Leu	5,81	5,72	5,36	5,94	6,18	6,21	6,08
Фенилаланин	Phe	3,99	4,32	4,00	4,20	4,31	4,19	4,42
Треонин	Thre	3,61	3,67	3,60	3,76	3,12	3,82	3,55
Серин	Ser	6,82	6,65	6,47	7,22	6,35	6,98	6,71
Глицин	Gly	4,79	5,51	4,35	5,70	5,83	5,62	5,23
Валин	Val	3,82	3,82	3,92	4,58	4,19	4,35	4,10
Аланин	Ala	2,80	2,51	3,02	4,13	3,81	3,76	3,62
Цистеин	Cys	3,48	3,10	3,15	3,28	3,61	3,46	3,01
Метионин	Met	3,69	3,16	2,70	4,03	3,82	3,76	3,89
Аспарагиновая кислота	Asp	9,30	8,94	9,57	9,12	9,40	9,46	9,32
Пролин	Pro	5,35	5,27	5,36	4,42	5,41	4,48	4,69
Глутаминовая кислота	Glu	15,73	16,09	15,14	14,22	14,14	14,32	13,88
ИНАК EAAI		82,11	78,46	76,20	82,74	81,44	84,07	81,07

Wpływ Miwału i Krezacyny na cechy morfologiczne, plon słomy i ziarna, oraz na zawartość bialka i jego skład aminokwasowy w ziarnie gryki (Fagopyrum esculentum Moench).

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Streszczenie

Badano wpływ dwóch nowych regulatorów wzrostu – Miwału i Krezacyny na grykę. Zastosowanie tych preparatów nie miało wplywu na przebieg faz rozwojowych roślin, nie róznicowało równiez wyraźnie cech biometrycznych. Oba regulatory obnizyly plon słomy, a Krezacyna zastosowana w najnizszej koncentracji podniosla nieco plon ziarna.
Miwal i Krezacyna nie wpłynęły istotnie na zawartość białka ale zmieniły w nim wyraźnie stosunki ilościowe aminokwasów egzogennych – wzrosła zawartość izoleucyny, leucyny, waliny, metioniny i cysteiny, a zmniejszyła się zawartość lizyny i argininy.

VPLIV MIVALA IN KREZACINA NA RAST IN RAZVOJ RASTLIN AJDE, NA PRIDELEK SLAME IN ZRNJA IN NA KEMIČNO SESTAVO.

Izvleček

Raziskovan je bil vpliv Mivala in Krezacina, dveh novih regulatorjev rasti. Avtorji niso mogli ugotoviti značilnega vpliva omenjenih regulatorjev rasti na rast in razvoj ajde, je pa nastala določena sprememba aminokislinske sestave beljakovnih zrn.

The influence of Mival and Krezacyne on the morphological features, straw and grain yield and protein content and its amino-acid composition in buckwheat grain (*Fagopyrum esculentum* Moench)

Summary

The influence of two new growth regulators - Mival and Krezacyne on buckwheat were studied.

The application of these preparations had no influence on the progress of plant growth phases and caused no obvious differences in the biological features. Both regulators decreased the yield of straw and Krezacyne used at the lowest concentration slightly increased the grain yield. Mival and Krezacyne did not substantially influence the crude protein content, but changed considerably the exogenic amino-acid ratios - the content of isoleucine, leucine, valine, methionine and cysteine was increased and the content of lysine and arginine decreased.

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Изменчивость активности флавонолоксидазы в разных органах тетраплоидной гречихи посевной.

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Ключевые слова: флавоноиды, онтогенез, автофертильность.

Key words: flavonoids, ontogeny, autofertility.

Введение

Гречиха является одним из богатых источников растительных фенолов и, в частности, флавоноидов, – органических соединений, среди которых выделено много веществ, имеющих пищевое и лекарственное значение для человека (1). Из этих веществ наиболее известен рутин, изучение которого завершилось разработкой регламентов его выделения из гречихи (2, 3). Однако в данной статье мы хотим привлечь внимание к флавоноидам гречихи в другом аспекте: с точки зрения их значимости в онтогенезе гречихи и их связи с функционированием системы размножения (4–8).

Недавно у гречихи был выделен и описан фермент, названный флавонолоксидазой, способный окислять флавонолы в отсутствие перекиси водорода (8, 9). Было показано, что в отличие от пероксидазы, широко распространенной в растениях и катализирующей окисление многих фенольных соединений (флавонолы, флавоны, халконы и др.), флавонолоксидаза гречихи обладает узкой субстратной специфичностью: она окисляет только флавонолы, незамещенные в положении C_3 и имеющие незамещенные 3′, 4′ – диоксигруппировки в кольце В. При этом уровень активности флавонолоксидазы (АФО) значительно ниже и более соответствует нормальным физиологическим процессам, по сравнению с очень высоким уровнем активности пероксидазы. Низкий уровень АФО в растениях и узкая субстратная специфичность флавонолоксидазы позволили авторам предположить более вероятное, по сравнению с пероксидазой, участие этого фермента в метаболизме флавонолов в нормальных физиологических условиях. Авторы предположили также существенное участие флавонолоксидазы в процессах онтогенеза, связанных с особенностями системы размножения гречихи. Эти предположения были подтверждены и развиты в дальнейших исследованиях (10).

Изучение изменчивости содержания флавонолов и активности флавонолрасщепляющих ферментов (гликозидазы, пероксидазы и флавонолоксидазы) привело авторов к заключению, что взаимодействие этих процессов играет важную регулирующую роль при переходе растений гречихи от бутонизации к фазе опыления-оплодотворения и основным регулятором может быть именно АФО. В цветках гречихи флавонолрасщепляющие ферменты представлены только флавонолоксидазой, тогда как все другие органы растения содержат их полный набор. По-видимому, именно АФО регулирует динамику накопления и расходования флавоноидов в период бутонизации и цветения и при этом колебания АФО закономерно связаны с разными типами цветков, т. е. со структурой локуса S.

Отметим здесь три установленные закономерности, которые явились предпосылками для нашей попытки использовать АФО в селекции на повышение автофертильности: 1) незначительные различия по АФО в репродуктивных органах между короткостолбчатыми (К) и длинностолбчатыми (Д) растениями исходной популяции, из которой были выделены автофертильные $К^+$ и $Д^+$ формы; 2) значительные различия по АФО в репродуктивных органах между автоферильными $К^+$ и $Д^+$ формами, а именно: АФО у $Д^+$ форм ниже, чем у $К^+$ форм; 3) неуклонное закономерное понижение АФО от К и Д форм исходной популяции к самофертильной $Д^+$ форме вплоть до полного отсутствия АФО у $Г_Д{}^+$ – самофертильной гомостильной формы с длинными равновысокими тычинками и пестиками (10).

Материал и методы

Объектом нашего исследования является мономорфная длинностолбчатая форма тетраплоидной гречихи, названная нами ГМ, селектируемая на повышение автофертильности (11, 12). Задача создания автофертильной сортовой популяции тетраплоидной гречихи, которую мы поставили перед собой, является очень сложной и в решении этой задачи мы стараемся использовать не только прямой отбор высоко автофертильных растений из лучших семей, наиболее плодовитых при самоопылении и переопылении между собой, но и вспомогательные методы: отбор по мейозу (а именно: по проценту измененных тетрад микроспор), отбор при выращивании растений в разных условиях (тепличные и полевые условия), статистические методы, позволяющие определить наследуемость автофертильности (11, 12).

Настоящее исследование исходит из задачи использовать отбор по АФО как один из способов повышения автофертильности и является первым этапом в решении этой задачи – изучением индивидуальной и посемейной изменчивости АФО в разных органах растений формы ГМ: в семенах, семядольных листьях и цветках, т. е. в разные периоды онтогенеза.

Ранее мы показали широкую изменчивость формы ГМ по содержанию рутина (посемейные средние проценты рутина в листьях варьировали от 0,8 до 3,7%) и более высокое содержание рутина у Д-растений исходной сортовой популяции Большевик 4, по сравнению с К-растениями этого сорта (13). Мы показали также наличие положительной корреляции между среднесемейным процентом рутина в листьях, с одной стороны, и с другой стороны: среднесемейными значениями массы зрелого растения ($r = 0,80 \pm 0,15$), числа цветков на растении ($r = 0,78 \pm 0,16$), общей суммы цветков, завязей и выполненных плодов на растении ($r = 0,65 \pm 0,24$) (14).

Таким образом, можно предполагать, что на тетраплоидном уровне так же, как на диплоидном, метаболизм флавонолов и связь его с продуктивностью растений достаточно существенны, чтобы можно было начать поиски таких связей, которые затрагивают атофертильность.

Изменчивость АФО изучали в покоящихся семенах (плодах), семядольных листьях 10-дневных проростков и в цветках, Семена и проростки изучались в 50 семьях, а цветки – в 14 из этих же 50 семей. Выборки включали в каждой семье: 24 индивидуальных анализа семян, 5 индивидуальных анализов проростков, 15 индивидуальных растений (по 10 цветков с растения) при анализе цветков.

Приготовление ферментных экстрактов.

Семена (сухие плоды) настаивали с 5 мл 0,1 М фосфатного буфера pH 5,9 в течение 2 час., затем сливали жидкость и использовали ее для определения АФО.

Семядольные листья 10-дневных проростков растирали с порошком полиамида и с 3 мл 0,1 М фосфатного буфера pH 5,9, профильтровывали гомогенат и использовали фильтрат для определения АФО.

По 10 цветков с каждого растения растирали с порошком полиамида и с 5 мл 0,1 М фосфатного буфера pH 5,9. Гомогенат профильтровывали и фильтрат использовали для определения АФО.

Определение АФО.

При работе с семенами и проростками брали 2,6 мл ферментного экстракта, добавляли 0,4 мл 0,33 мМ спиртового раствора кверцетина и измеряли уменьшение оптической плотности (ОП) при 375 нм за определенный промежуток времени.

При работе с цветками брали 1,6 мл 0,1 М фосфатного буфера pH 5,9, прибавляли 1 мл экстракта из цветков и 0,4 мл 0,33 мМ спиртового раствора кверцетина и также измеряли уменьшение ОП при 375 нм за определенный промежуток времени.

Единицы, в которых приводятся данные по АФО для семян: ОП/семя · 20 час; для проростков: ОП/семядольный лист · 1 час; для цветков: ОП/10 цветков · 10 мин. Для лучшей сравнимости средних величин АФО в разных органах был сделан также пересчет в ОП/ 1 орган · 1 час.

При статистической обработке данных использовали двухфакторный дисперсионный анализ и определяли показатели силы влияния изученных факторов (фактор А – орган растения, фактор В – суммарный генотип семьи) на изменчивость АФО (15).

Экспериментальная часть и обсуждение.

Основные результаты исследований представлены на Рис. 1 и в таблицах 1, 2, 3.
Рис. 1 представляет распределения средних семейных значений АФО в разные периоды онтогенеза: в покоящихся семенах (плодах), в семядольных листьях 10-дневных проростков и в цветках во второй половине периода цветения.
Табл. 1 дополняет данные рис. 1 показателями изменчивости средних семейных значений АФО.
Размах изменчивости средних семейных АФО более широкий в семенах и в проростках, по сравнению с цветками, о чем говорят соотношения min : max и значения внутрисемейных коэффициентов вариации CV. Однако межсемейные CV невелики (13 ÷ 25%). В проростках межсемейный CV наибольший и равен 25 ± 3%.
Абсолютные значения АФО в разных органах более точно можно сравнить при пересчете на ОП/1 орган · 1 час.; в этом случае получается соотношение по АФО в семенах : в проростках : в цветках = 0,003 : 0,300 : 0,209 = 1 : 100 : 70.

Таблица 1. Table 1.

Изменчивость АФО у формы ГМ в разных органах растения.

Flavonoloxidase activity (АФО) variation in different plant organs of GM form.

	Семена Seeds	Проростки Seedlings	Цветки Flowers
Число семей Number of families	50	50	14
M_{Σ} Средняя для всех семей Average of all families	0,066 ± 0,001	0,300 ± 0,009	0,349 ± 0,012
Пределы M_i Limits of M_i	0,041 ÷ 0,086	0,157 ÷ 0,425	0,290 ÷ 0,416
min : max	1 : 2,1	1 : 2,7	1 : 1,4
Внутрисемейные CV (%)* Intrafamily CV (%)	46 ÷ 92	10 ÷ 109	19 ÷ 50
Межсемейные CV (%) Interfamilies CV (%)	14 ± 1	25 ± 3	13 ± 2

*CV – coefficient of variation

При анализе семян были отмечены нулевые значения АФО у 19 из 50 семей, по 1–2 семени в каждой семье.
Следует отметить, что при постановке опыта были возможны колебания условий, которые могли повлиять на изменчивость индивидуальных показателей АФО. Поэтому в каждой выборке семян, проростков и цветков брали в анализ одновременно по одному представителю каждой семьи. Это дает возможность считать изученные выборки семей достаточно репрезентативными.

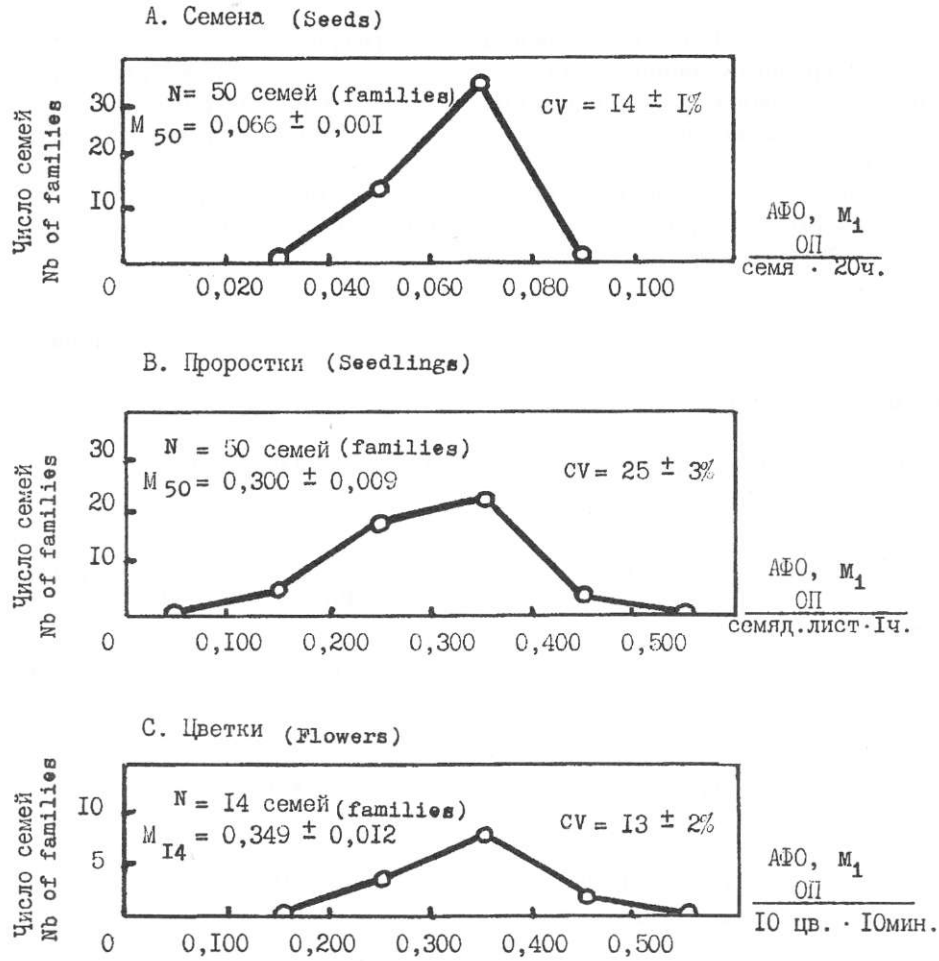

Рис. I. Распределение семейных средних (M_1) по АФО.

Fig.I. Distributions of family averages (M_1) of flavonoloxidase activity (АФО):

A - $\frac{\text{optical density}}{\text{seed} \cdot 20\ \text{hr}}$;

B - $\frac{\text{optical density}}{\text{seed-leaf} \cdot 1\ \text{hr}}$;

C - $\frac{\text{optical density}}{10\ \text{flowers} \cdot 10\text{min}}$.

Генотипы изучавшихся семей довольно близки между собой. Одна из семей селектируемой формы ГМ была высеяна на изолированном участке в 1987 г. для размножения внутри себя. Из 69 переопылившихся растений было отобрано 50 лучших по продуктивности и эти 50 растений дали семена для наших исследований по АФО. С этих же растений были взяты семена для дальнейшей селекции на повышение автофертильности. В частности, у 14 семей из 50 мы изучали в 1989 г. содержание АФО в цветках, а также соотношение типов цветков в онтогенезе и продуктивность.

Ранее мы отмечали в своих работах, что селекция на повышение автофертильности у Д-формы тетраплоидной гречихи ведет к повышению доли равностолбчатых (Р) цветков с короткими тычинками и пестиком, а также к увеличению доли цветков с уменьшенной разницей в длине тычинок и пестика (Д ~ Р). Мы наблюдали изменчивость соотношения этих типов цветков в пределах одного растения и одной и той же семьи в разные периоды онтогенеза и в разных условиях выращивания (11, 12, 16). В данном случае те семьи, которые анализировались по АФО, имели большой размах изменчивости и по соотношению типов цветка. В целом за весь период онтогенеза преобладали типичные Д-цветки: их было в среднем 57 ± 3% и посемейные средние изменялись от 35 до 70%. Цветков типа Д ~ Р было в целом 19 ± 2% с колебаниями по семьям от 12 до 32%. Цветки третьего типа, т. е. Р, составляли 24 ± 2% от общего числа изученных, а колебания по семьям были от 16 до 38%.

Таблица 2. Table 2.

Влияние факторов А (семена или семядольные листья проростков) и В (генотип семьи) на изменчивость величины АФО у 50 семей формы ГМ*.

Analysis of variance: 50 families
A – plant organ; B – family genotype.

	A	B	AB	X	Z	Y
η^2	55,3	5,9	5,6	66,8	33,2	100
	±0,0	±1,2	±1,2	±2,4		
ν	1	49	49	99	1312	1411
F	2193	4,7	4,5	26,7		

* η^2 – показатель силы влияния (per cent of influence)

Таблица 3. Table 3.

Влияние факторов А (семена, семядольные листья, цветки) и В (генотип семьи) на изменчивость величины АФО у 14 семей формы ГМ*.

Analysis of variance: 14 families
A – plant organ; B – family genotype.

	A	B	AB	X	Z	Y
η^2	58,7	3,5	8,7	71,1	28,8	100
	±0,1	±0,6	±1,3	±2,1		
ν	2	13	26	41	547	588
F	556,7	5,2	6,3	32,8		

* η^2 – показатель силы влияния (per cent of influence)

Возможно, колебания в соотношении типов цветка также могли влиять на характеристику семей по АФО.
В будушем мы продолжим изучение связей между АФО, типом цветка и продуктивностью у растений формы ГМ. В настоящем материале некоторые корреляции были выявлены при коррелировании семейных средних. Была отмечена отрицательная корреляция между уровнями АФО в исходных семенах и в цветках: $r = -0{,}5 \pm 0{,}2$; $t = 2{,}5$; $B = 0{,}95$. Отрицательная корреляция была отмечена также между уровнем АФО в цветках и такими важными показателями, как среднее количество кистей на растении ($r = -0{,}7 \pm 0{,}2$) и количество выполненных зёрен ($r = -0{,}5 \pm 0{,}2$). Все эти корреляции установлены не между показателями индивидуальных растений, а между средними семейными величинами соответствующих признаков для 14 семей формы ГМ.

Пока трудно сказать, насколько повторимы полученные нами результаты в зависимости от объекта и условий, могут ли эти данные быть использованы в селекции на повышение автофертильности. Однако важно отметить, что дисперсионный анализ двух комплексов данных, полученных нами при изучении АФО, показал статистически достоверное влияние изученных факторов: А – орган растения, В – генотип семьи на изменчивость АФО в форме ГМ (табл. 2, 3).
В табл. 2 представлены результаты дисперсионного анализа 1-го комплекса данных по АФО: в семенах и проростках 50 семей формы ГМ (2 градации фактора А).
В табл. 3 представлены результаты дисперсионного анализа данных по АФО в другом комплексе: не только в семенах и проростках, но и в цветках (3 градации фактора А); в этот комплекс вошли данные только по 14 семьям из 50.
Отобранные 14 семей происходили от наиболее продуктивных растений. Группа из 69 переопылявшихся между собой растений формы ГМ, из которой были взяты родители для 50 семей нашего опыта, имела в среднем 206 ± 14 выполненных зерен на растении ($CV = 114 \pm 10\%$) с массой 1000, равной $31{,}3 \pm 0{,}3$ г, а группа родителей для 14 семей имела в среднем 369 ± 14 выполненных зерен ($CV\ 14 \pm 3\%$) с массой 1000, равной $31{,}9 \pm 0{,}5$ г.
Из таблиц 2 и 3 мы видим, что оба наши комплекса данных по АФО выявили вляние стадии онтогенеза (А – орган растения: семя, семядольный лист, цветок) и генотипа семьи (В) на изменчивость АФО. Выявилось также достоверное влияние взаимодействия этих факторов. Отсюда можно сделать вывод, что резервы селекции по АФО в нашем материале имеются.
В дальнейших исследованиях мы продолжим изучение связей между уровнем АФО в разных органах формы ГМ и ее автофертильностью.

Заключение

Мы изучили изменчивость активности флавонолоксидазы (АФО) в разных органах растений мономорфной длинностолбчатой формы тетраплоидной гречихи (ГМ), селектируемой на повышение автофертильности.
Данная работа является начальным этапом на пути применения показателя АФО в селекции на повышение автофертильности. Исследования показали существенное влияние таких факторов, как орган растения и генотип семьи на изменчивость АФО. Установлены некоторые коррелятивные связи между показателями АФО и продуктивности.

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VARIABILNOST AKTIVNOSTI FLAVONOLOKSIDAZE V RAZLIČNIH ORGANIH TETRAPLOIDNE AJDE

Izvleček

Raziskovana je bila aktivnost flavonoloksidaze v različnih organih monomorfne pin oblike tetraploidne ajde, selekcionirane za povečano avtofertilnost. Ta raziskava je začetek uporabe aktivnosti flavonoloksidaze pri izbiri za povečano avtofertilnost. Ugotovljena je značilna povezava med flavonoloksidazno aktivnostjo in pridelkom ajde.

Variation of flavonoloxidase activity in different organs of tetraploid buckwheat.

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Summary

The variability of flavonoloxidase activity (AFO) was studied in different plant organs of monomorphic pin-form of tetraploid buckwheat (GM) wich was selected for increasing of autofertility. This investigation is the first step in the way of using of AFO in selection for increasing autofertility. A significant influence of factors such as plant organ and family genotype on AFO variability was demonstrated. Some correlations were established between AFO and productivity.

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МОСКОВСКОЕ ОБЩЕСТВО ИСПЫТАТЕЛЕЙ ПРИРОДЫ: ДОКЛАДЫ МОИП 1987, ОБЩАЯ БИЛОГИЯ, МОРФОЛГИЯ И ГЕНЕТИКА ПРОЦЕССОВ РОСТА И РАЗВИТИЯ, МОСКВА 1989

СЕКЦИЯ ГЕНЕТИКИ

ТЕЗИСЫ ДОКЛАДОВ, ПЯТОГО ВСЕСОЮЗНОГО СИМПОЗИУМА ПО ФЕНОЛЬНЫМ СОЕДИНЕНИЯМ, ТАЛЛИН, 22-24 СЕНТЯБРЯ 1987 ГОДА

ERRATA - POPRAVKI

In the paper of M. Knežević (Fagopyrum 9, 1989) on the top of p. 51 is missing: The applied herbicides and their combinations were not phytotoxic for buckwheat, while they affected variously the present weed species (Tables 2 and 3). Preemergence chemicals Dual 500 EC and Bravo, alone or in combinations with Deherbane A and Dicofluid MP Combi, affected on the lessening of Monocotyledoneae number in agrophytocenosis, and especially of the Echinochloa crus galli individuals. These herbicides gave high efficacy coefficients for Monocotyledoneae, ranging from 98.7% in combination of Dual 500 EC + Deherban A (3 + 1.5 l/ha) to 100% by application of Bravo chemical (5 l/ha) and combination of Bravo + Deherban A (5 + 1.5 l/ha). The postemergence chemical Targa, at a rate of 1.5 l/ha, killed on the 15th day of application all grasses in buckwheat, showing thus excellent efficacy in killing of grassy weeds.

The herbicides Dual 500 EC and Bravo, as well as combinations Dual 500 EC + Deherban

In the paper of A.N. Bočkarev, L.P. Bočkareva (Fagopyrum 9, 1989) on the p. 61 second row under the Table 2 read: $Y = A : (1 + e^{a + BX})$

INSTRUCTIONS FOR AUTHORS

"Fagopyrum" accepts
-scientific papers,
-information and bibliographies.

SCIENTIFIC PAPERS. Submitted papers are refereed by two reviewers. Papers submitted through a member of the advisory committee will be refereed by one additional reviewer. FAGOPYRUM will follow the system of ISI units (Systeme International d'Unitees).

In order to expedite publication and to reduce costs, the editor prefers "camera ready" manuscripts supplied by the authors. Your manuscript will be letterset or printed on a laser printer in a style similar to that in recent editions of Fagopyrum. In this case, corrections will not be possible after receipt of papers. Please make sure the text contains no typing mistakes. However, corrections will be needed if so proposed by a referee.

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The name(s) of author(s) and page number and/or figure number should be clearly marked on the back of all diagrams, photographs etc. Illustrations should be planned to fit either a half or the full width of the page. Black and white photographs should be of good quality and contrast, lettering or marking should be of the same style and quality as in recent volumes of "Fagopyrum". If this is not possible for the author, photographs should be sent without lettering; but lettering and marks should be clearly positioned on a copy. Colour photographs for black and white printing. Colour photographs can exceptionaly be printed as black/white photographs, although the quality is inferior, especially if there are no good contrasts on the photograph, or if contrasts are in green/red tones. Avoid red backgrounds behind green objects and use white or other light background.

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The Editor.

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