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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.

Front page photo: Bread made with varying amounts of buckwheat flour and pasta produced with varying amounts of buckwheat flour. (See paper of G. Bonafaccia and I. Kreft, pp. 35-42).

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Fagopyrum 14 (1994): 3 - 6

α–Amylase inhibitor in buckwheat seed

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Abstract

This paper presents evidence for the occurrence of an α-amylase inhibitor in buckwheat seed. The buckwheat inhibitor exhibited inhibitory activity against α-amylase from human saliva and α-amylase from porcine pancrease, but less or substantially no inhibitory activity against α-amylase from *Bacillus subtilis* and β-amylase from sweet potato. Gel filtration chromatography of buckwheat extract on Toyopearl HW-40 showed that the α-amylase inhibitor emerged as a single peak. The present findings suggest that α-amylase inhibitor in buckwheat may be a protein-like substance.

Inhibitor α-amilaze v semenih ajde

V delu so prikazane ugotovitve o inhibitorju α-amilaze v semenih ajde. Ta inhibitor zavre aktivnost α-amilaze sline ljudi in α-amilaze trebušne slinavke prašičev, ima pa manj oziroma praktično nima inhibitornega učinka na α-amilazo *Bacillus subtilisa* in β-amilazo sladkega krompirja. Pri gelsko filtracijski kromatografiji ekstrakta ajde na Toyopearlu HW-40 je inhibitor α-amilaze dal enojen vrh. Na osnovi ugotovitev avtorji sklepajo, da je inhibitor α-amilaze pri ajdi lahko beljakovinam podobna snov. (Prevod uredništva).

Introduction

Buckwheat (*Fagopyrum esculentum* Moench) is an important crop in some regions of the world. There are a variety of products made from buckwheat flour or groats around the world. On the other hand, it is well known that inhalation or ingestion of flour or products of buckwheat provokes severe allergic symptoms in some sensitive persons (Perlman 1980). However, limited work has been reported relating to allergy to buckwheat. In addition, studies on the allergen of buckwheat are the subject of controversy (Yanagihara 1980, Yano et al. 1989, Kondo et al. 1993).

Protein protease inhibitors are widely distributed among legumes and cereals (Linear and Kakade 1980, Rackis and Gumbmann 1981). The protease inhibitors have been extensively investigated because of the adverse effects they may have on human nutrition. We have presented evidence for the occurrence of a protein protease inhibitor in buckwheat seed (Ikeda and Kusano 1978) and discussed some properties of the inhibitor (Ikeda et al. 1986 and 1991). On the other hand, protein amylase inhibitors are also found among some legumes and cereals (Garcia-Olmedo et al. 1987). In contrast to protease inhibitors, amylase inhibitors are not well understood. Recently, each α-amylase inhibitor of wheat and barley has been identified as a major allergen associated with baker's asthma disease (Berber et al. 1989, Gomez et al. 1990). Thus a relationship between enzyme inhibitors and food allergy is the subject of intense investigation.

It remains unknown whether or not there is an amylase inhibitor in buckwheat seed. There is a possibility that inhibitors of enzymes such as amylase or trypsin, if any, might be closely associated with allergy to buckwheat. The present study investigates evidence for the occurrence of an α-amylase inhibitor in buckwheat seed.

Materials and analytical methods

Materials

Fresh buckwheat flour obtained by commercially-milling the seed was examined in this study. Three different kinds of other commercial cereal foods were also examined: dehulled barley grain; wheat hard flour; and corn meal. Three different kinds of amylase (EC 3.2.1.1.) preparations were obtained from Sigma Chemicals Co. (USA): α-amylase from porcine pancreas (28 units/ mg solid); α-amylase from *Bacillus subtilis* (2500 units/mg protein) and β-amylase from sweet potato (965 units/mg protein). Salivas from three healthy students were used as a human α-amylase source: each saliva collected was diluted 2-times with 0.1M phosphate buffer (pH 6.9), and immediately used for enzyme inhibitory assay. Soluble starch was obtained from Nacalai Tesque Inc. (Japan). Pepsin (EC 3.4.23.1) from porcine stomach

mucosa (3500 units/mg protein) was obtained from Sigma Chemicals Co. Toyopearl HW-40 was a product of Toyo Soda MFG, Co., Ltd. (Japan). All other chemicals used were of analytical grade.

Extraction

Buckwheat flour was extracted with 8-fold volume (v/w) of 0.9% sodium chloride solution with stirring for 1 hr at room temperature. After extraction, the suspension was routinely heated at 80°C for 10 min in order to inactivate endogenous amylase activity present. The heated suspension was then centrifuged at 3,000 rpm for 10 min. The supernatant obtained was assayed for amylase inhibitory activity.

Analytical methods

Amylase activity was assayed by the colorimetric procedure with 3,5-dinitrosalicylic acid (Bernfeld 1955). Inhibitory activity against amylase was assayed by the same procedure used in the detection of protease inhibitors described previously (Ikeda and Kusano 1978): 0 to 15 μl aliquots of buckwheat heated extract were preincubated with 20 μl of enzyme solution in a total volume of 70 μl. After the addition of 1.0 ml of 1% soluble starch solution in 0.1M phosphate buffer (pH 6.9) into the reaction mixture, the remaining enzyme activity was assayed at 37°C for 5 min. The enzyme reaction was terminated by the addition of 2.0 ml of 3,5-dinitrosalicylic acid solution. The control mixture was prepared by replacing the extract with 0.9% sodium chloride solution. Relative inhibitory activity (RIA) was expressed as per cent inhibition to control assay using the equation RIA (%) = ((WO-WI)/WO) x 100, where WI and WO are enzyme activities with or without inhibitor, respectively. Protein was assayed by the method of Bradford (1976).

Chromatography

About 3 ml aliquot of buckwheat native extract was applied to a Toyopearl HW-40 column (65 cm x 1.8 cm ϕ), pre-equilibrated against 0.1 M Tris-HCl buffer (pH 7.2). Elution was performed with this buffer, and 4.0 ml-fractions were collected.

Pepsin digestion of amylase inhibitor

An α-amylase inhibitor fraction, obtained by gel filtration chromatography of buckwheat extract on Toyopearl HW-40, was pooled. A 1.0 ml aliquot of the active fraction pooled was incubated with a solution containing 50 μg pepsin in 0.1M HCl-KCl buffer (pH 2.0) for 37°C. After incubation with pepsin, the remaining α-amylase inhibitory activity was assayed.

Results and discussion

Figure 1 shows inhibitory activity of buckwheat extract against α-amylase activities of three different human salivas. Buckwheat extract exhibited inhibitory activity against three human salivary α-amylases. Figure 2 shows inhibitory activities of buckwheat extract against various amylases. The highest enzyme-inhibitory activity was found with human salivary α-amylase ((A) in Figure 2) among amylases examined. Buckwheat extract also exhibited inhibitory activity against porcine pancreatic α-amylase ((B) in Figure 2), but less or substantially no inhibitory activity against α-amylase from *Bacillus subtilis* ((C) in Figure 2) and β-amylase from sweet potato ((D) in Figure 2).

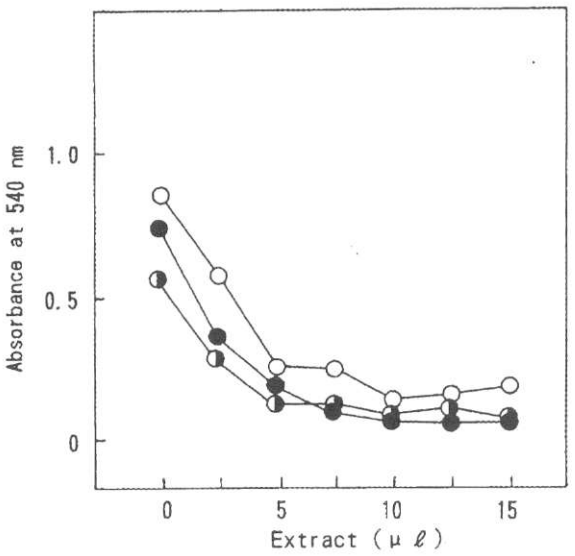

Figure 1: Effect of buckwheat extract on the activities of human salivary α-amylase.

Figure 3 shows inhibitory activities against human salivary α-amylase of extracts of several cereal flours, including buckwheat flour. The extract of wheat flour exhibited high enzyme-inhibitory activity. The enzyme-inhibitory activity of the extract of buckwheat flour was rather lower than that of the extract of wheat flour. Substantially no inhibitory activity was found with barley flour under the conditions employed. Garcia-Olmedo et al. (1987) have described that several types of barley α-amylase inhibitors, i.e. CMb' and the tetrameric type of CMa, are active against

α-amylase from the insect *Tenebrio molitor* but show no effect against salivary α-amylase, agreeing with the present finding (Figure 3). In addition, no inhibitory activity against salivary α-amylase was found with corn meal (Figure 3).

Figure 4 shows the chromatographic elution profile of buckwheat extract on Toyopearl HW-40. Inhibitory activity against salivary α-amylase emerged as a single peak. In addition, inhibitory activity against salivary α-amylase was eluted at the same position as inhibitory activity against pancreatic α-amylase. This finding suggests that inhibitory activity against salivary α-amylase may be identical with inhibitory activity against pancreatic α-amylase. Furthermore, inhibitory activity against salivary α-amylase was higher than inhibitory activity against pancreatic α-amylase (Figure 4).

Susceptibility of buckwheat α-amylase inhibitory activity to peptic action was examined: a fraction containing buckwheat α-amylase inhibitor, obtained by gel filtration chromatography (Figure 4), was incubated with pepsin. After incubation with pepsin for 30 min, about 70% of the original enzyme-inhibitory activity of its fraction disappeared (data not shown), suggesting that the α-amylase inhibitor is a protein-like substance. In conclusion, our findings present evidence for the occurrence of a protein-like α-amylase inhibitor in buckwheat seed. Further characterization of the α-amylase inhibitor in relation to allergy is underway in our laboratory.

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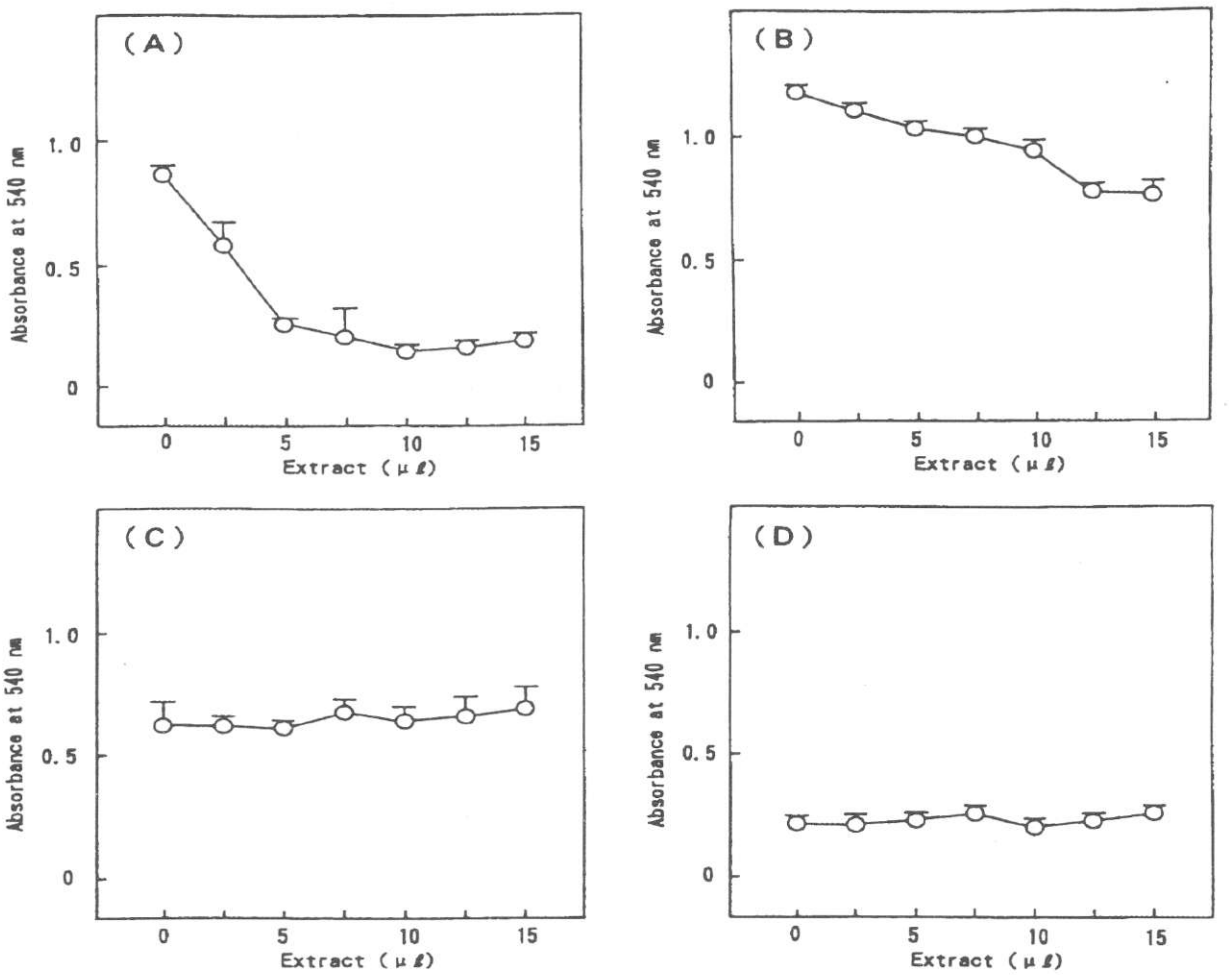

Figure 2: Effects of buckwheat extracts on the activities of various amylases: (A) human salivary α-amylase, (B) porcine pancreatic α-amylase, (C) α-amylase from *Bacillus subtilis* and (D) β-amylase from sweet potato.

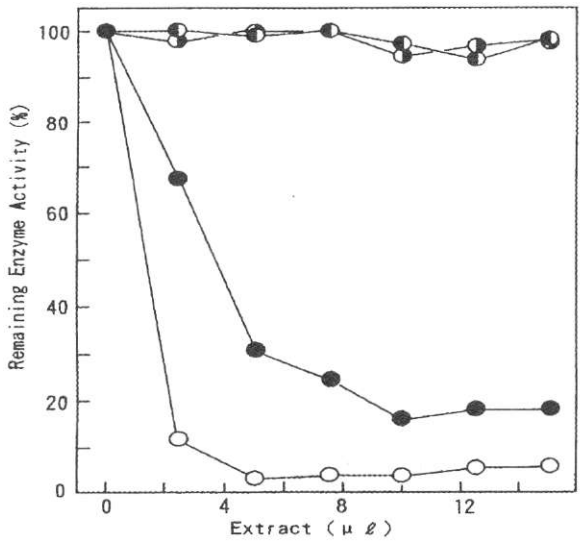

Figure 3: Effects of various foods on the activity of human salivary α-amylase: ● buckwheat flour, ○ wheat flour, ◑ barley flour and ◐ corn meal.

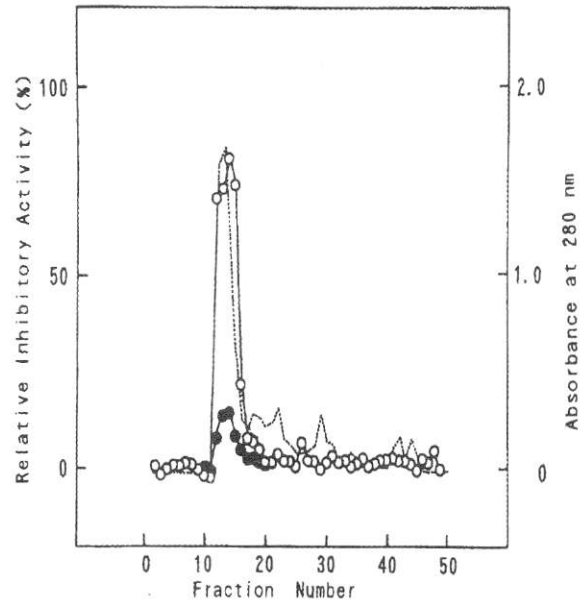

Figure 4: Gel filtration chromatography of buckwheat extract on Toyopearl HW-40: ○ inhibitory activity against human salivary α-amylase, ● inhibitory activity against porcine pancreatic α-amylase and ..... absorbance at 280 nm.

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Protoplast fusion in buckwheat: preliminary results on somatic hybridization*

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Key words: *Fagopyrum*, protoplast fusion, DNA analysis, somatic hybrid

Abstract

Mesophyll protoplasts of *Fagopyrum esculentum* were fused by PEG-mediated fusion with hypocotyl protoplasts of *F. tataricum*, serving as the hauler. The fusion experiments were aimed at the elaboration of simple procedures for overcoming breeding barriers in buckwheat. A simple two-step preselection method using the intolerance of mesophyll protoplasts to the fusion procedure and the appearance of *F. tataricum* calli could be established. The hybrid nature of calli obtained was verified by RFLP analysis. The callus clones analysed predominantly expressed one parental nuclear DNA with one callus expressing bands specific to both parents. Use of a mtDNA probe also revealed a predominance of one parental mtDNA fragment, as well as the presence of fragments of the other parent in low proportions, and novel bands. All the calli analysed carried one parental chloroplast DNA type.

Fuzija protoplastov pri ajdi: predhodni rezultati somatske hibridizacije

Mezofilni protoplasti *Fagopyrum esculentum* so bili združevani s PEG metodo s hipokotilnimi protoplasti *Fagopyrum tataricum*. Namen poskusov je bil izdelati metodo, ki bi omogočila združevanje genomov obeh ajd. Razvili so enostavno dvo-stopenjsko metodo, ki je temeljila na odbojnosti med seboj enakih mezofilnih protoplastov navadne ajde in ločljivosti po videzu kalusov tatarske ajde. Z RFLP analizo so proučevali poreklo regeneriranih kalusov. Analizirani kloni so pretežno izkazovali starševsko obliko, en kalus je izražal RFLP črte obeh vrst. Analiza z mtDNK sondami je tudi pokazala poreklo pretežno le od enega starša, v majhnem obsegu pa tudi prisotnist dela DNK drugega starša ter pojav novih črt. Analiza kloroplastne DNK je pokazala, da so vsi kloni vsebovali le po en starševski tip. (Prevod uredništva).

Introduction

Common buckwheat (*Fagopyrum esculentum* Moench) is not well-adapted to classical plant breeding due to its heteromorphic, sporophytic incompatibility system, so its yield today is still low and variable, and it more resembles a wild plant than a cultivated crop (Kreft 1989). However, common buckwheat is recognized for its very high nutritional value, particularly for its extraordinary high protein quality (Eggum 1980, Javornik et al. 1981), which makes it a highly valuable supplement in human nutrition. It also shows other excellent properties, such as a short vegetative period, strong competition against weeds, and is not susceptible to most cereal diseases.

Somatic hybridisation, which offers a unique and simple way to transfer genes between sexually incompatible species, has been suggested as a means of overcoming breeding barriers in buckwheat (Nešković et al. 1986).

The wild species, *F. tataricum*, sexually incompatible with common buckwheat, could provide genetic resources such as self-fertility, a high productivity and tolerance to harsh climatic conditions.

Recently, the isolation and culture of protoplasts from common buckwheat were described (Rumyantseva and Lozovaya 1987). Thereafter plant regeneration from common buckwheat protoplasts (Adachi et al. 1989, Gumerova 1991) and callus regeneration from tartary buckwheat protoplasts (Lachmann and Adachi 1990, 1991) were reported.

We describe herein fusion experiments designed to create somatic hybrids between common and tartary buckwheat. This is, to our knowledge, the first report of the production of somatic hybrids in the genus *Fagopyrum*.

Materials and methods

Plant material: Protoplasts of *F. tataricum* were isolated from hypocotyls of 5-6 days-old, etiolated seedlings, grown on 1/10 MS (Murashige and Skoog 1962) growth regulator-free medium. Protoplasts of *F. esculentum* cv. Sando-Soba and Miyazakizairai were

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isolated from shoot cultures maintained on MS medium containing 2 mg/l BA (benzylaminopurine), 0.2 mg/l IAA (3-indolacetic acid) or on MS medium devoid of growth regulators.

Protoplast isolation: Hypocotyl protoplasts were isolated following a modified method previously described (Lachmann and Adachi 1990). The plant material was preplasmolyzed in a solution containing 0.3 M sorbitol and 50 mM $CaCl_2 \cdot 2H_2O$. Enzyme digestion was performed in a solution containing MS macro- and micro-elements. The NH_4NO_3 concentration was reduced to 150 mg/l. Released protoplasts were washed twice in washing solution (0.5 M mannitol, 0.05 M $CaCl_2$). Purified protoplasts were washed in a buffer containing 0.15 M sorbitol, 0.03 M $CaCl_2$, 0.075 M KCl, 0.05 M Tris at pH 7 (Glimelius 1984). Green protoplasts were isolated from stems, petioles and young leaflets of aseptic shoot cultures. The material was finely sliced and treated as described for the hypocotyls.

Protoplast fusion: Prior to fusion, the protoplast density of both fusion partners was adjusted to the range of $1\text{-}5x10^5$ ppl/ml buffer solution. Equal volumes of protoplast suspensions were mixed and four drops were placed in a 3 cm plastic dish using a Pasteur pipette. The protoplasts were allowed to settle for at least five minutes before the fusion treatment. The fusion treatments were based on the methods of Hein et al. (1983), Glimelius et al. (1978) and Chand et al. (1988). Various combinations of PEG solutions and elution solutions were examined (Table 1). Two drops of PEG solution were added to the top of each drop of protoplast suspension. After 5 min incubation almost all the liquid was gently removed and the elution solution was added to the protoplasts adhering to the dish. Over a period of 5 min, culture medium was added until the bottom of the dish was covered. The liquid was gently swirled and left to stand for 2-3 min. The fluid was then removed and 0.5-0.7 ml of medium were added to the dish. In fusion protocol IV a serial elution described by Glimelius et al. (1978) was performed.

Protoplast culture: The protoplasts were cultured in a modified 8p medium (Kao and Michayluk 1975). The cultures were activated with a nurse callus from a vigorously growing callus culture of *F. esculentum* on top of an agarose block placed in the dish in order to avoid contact with protoplasts in the liquid film. All cultures were maintained in the dark at 20-25°C.

The cultures were fed with fresh, growth regulator-free medium containing 3% sucrose, starting seven days after fusion. Four to five weeks after culture initiation, small calli (about 0.1 cm) were transferred to fresh medium devoid of organic acids and the sugars of the protoplast medium containing only 3% sucrose, 2 mg/l NAA and 1 mg/l BA, solidified with 0.2% Gelrite (CPM). Simultaneously the calli were transferred to dim light conditions. After 2 weeks on this medium, the calli doubled their size and were transplanted onto regeneration media (SRM) containing MS macro- and micro-nutrients, 3% sucrose and various growth regulator combinations. The concentration of NH_4NO_3 was reduced to 825 mg/l (MS 1/2), and the concentrations of nicotinic acid, pyridoxin-HCl and thiamine-HCl were increased to 1, 1 and 5 mg/l, respectively. The calli were incubated under a photoperiod of 12 hr at 25°C. Alternatively, calli were placed on a similar medium containing 5 mg/l 2,4-D and 0.1 mg/l BA (CM5D) for 5-7 days in order to reinduce morphogenesis. After this culture period, they were also transferred to SRM media or maintained on CM1D medium.

Table 1: Fusion solutions employed in experiments with common and tartary buckwheat. (All solutions were filter sterilized.)

Ingredients		Fusion I	Fusion II	Fusion III	Fusion IV
PEG:					
PEG 6000 MW	(%)	25.00	25.00	25.00	-
PEG 1500 MW	(%)	-	-	-	40.00
$CaCl_2$	(M)	0.01	0.01	0.01	0.05
Mannitol	(M)	0.40	0.40	0.40	-
Glucose	(M)	-	-	-	0.30
pH		7.00	7.00	7.00	7.00
Elution:					
$CaCl_2$	(M)	0.05	0.05	-	0.10
Sorbitol	(M)	0.30	0.20	0.30	0.10
pH		7.00	7.00	5.80	7.00

Table 2: Modified 8p medium (PL). (All solutions were filter sterilized.)

Macro elements	(mg/l)	**Micro elements**	(mg/l)
NH_4NO_3	150	$MnSO_4 \cdot 4H_2O$	22.3
KNO_3	1900	$ZnSO_4 \cdot 7H_2O$	8.6
$CaCl_2 \cdot 2H_20$	600	H_3BO_3	6.2
$MgSO_4 \cdot 7H_2O$	300	KI	0.83
KH_2PO_4	170	$Na_2MoO_4 \cdot 2H_2O$	0.25
KCl	300	$CuSO_4 \cdot 5H_2O$	0.025
$FeSO_4 \cdot 7H_2O$	28	$CoCl_2 \cdot 6H_2O$	0.025
Na_2EDTA	37	**Sugars**	(g/l)
Vitamins	(mg/l)	Sucrose	0.125
Myo-inositol	100	Fructose	0.125
Nicotic acid	1	Ribose	0.125
Pyridoxin HCl	1	Xylose	0.125
Thiamine HCl	5	Mannose	0.125
Glycine	2	Rhamnose	0.125
Organic acid	(mg/l)	Cellobiose	0.125
Ascorbic acid	2	Sorbitol	0.125
Sodium pyruvate	5	Mannitol	0.125
Citric acid	10	Glucose	70.0
Maleic acid	10	**Plant growth regulators**	(mg/l)
Fumaric acid	10	α-Naphtaleneacetic acid	2
		6-Benzylamonopurine	1

Table 3: Results of fusion experiments utilizing different fusion procedures. The number of colonies was determined as the number of colonies larger than 0.1 cm in diameter, four weeks after fusion. Fusion products were determined by callus appearance. The number of fusion products is expressed as the percentage of calli recovered.

Experiment	No. of dishes	Fusion method	No. of colonies recovered	No. of colonies per dish	No. of calli recovered	No. of fusion products (%)
1	5	I	250	50	57	14 (25)
2	3	I	200	67	172	146 (65)
3	3	I	50	17	36	6 (17)
4	2	IV	106	53	38	4 (11)
5	2	IV	57	28	21	14 (67)
6	1	III*	43	43	12	1 (9)

*: Modified fusion method III with 30 % PEG

DNA isolation and digestion: Total DNA was isolated from the dry frozen callus (0.2-0.5 g.f.w.) of both parental species and putative fusion products. The isolation procedure was essentially as described by Honda and Hirai (1990), with only insignificant modifications. The DNA was digested with EcoRI. Restriction fragments were separated by overnight electrophoresis on 0.7% agarose gels containing ethidium bromide (0.5 µg/ml).

Southern hybridization: After electrophoresis, the DNA was visualized under a UV transilluminator and photographed. The gels were washed for 30 min in a denaturation solution (0.5 M NaOH, 1.5 M NaCl), followed for another 30 min in 1.5 M NaCl, 0.5 M Tris-HCl, pH 7. The DNA was transferred to Hybond N (Amersham, UK) with 20 x SSC overnight. Southern hybridization and detection were performed exactly as suggested by the manufacturer, using the ECL Hybridization Kit (Amersham, UK). The 5.8 kb rDNA encoding for two nuclear encoded ribosomal RNA genes (25 S and 18 S) from *Vicia faba*, as well as a 1.8 kb sequence encoding for the ATP α gene of *Beta vulgaris* mitochondria, and the *rbcL* gene

derived from rice chloroplasts were used as probes. All probes were kindly provided by Dr. T. Mikami, Hokkaido University, Japan.

Results

Fusion: Fusion was readily induced with all protocols tested, although the fusion frequency varied widely between experiments. Complete fusion products could be easily distinguished by the accumulation of chloroplasts around the nucleus of the hypocotyl protoplasts.

Among the fusion protocols tested, protocol I allowed the highest rate of colony formation followed by protocol IV (Table 3). Protocol III and elution without Ca^{++} left more viable protoplasts, and also allowed a high division frequency. In particular, the mesophyll protoplasts were not damaged to the same degree as after elution with high Ca^{++} concentration. Protocol I-III with 30% PEG produced a low rate of colony formation due to the strong adherence of protoplasts to the culture dish and aggregation of protoplasts, which led to browning and early death of the cells. Storage in a refrigerator (8°C) for several hours did not release the protoplasts from the dish, and it was necessary to handle multi-cell colonies rather roughly in order to separate them.

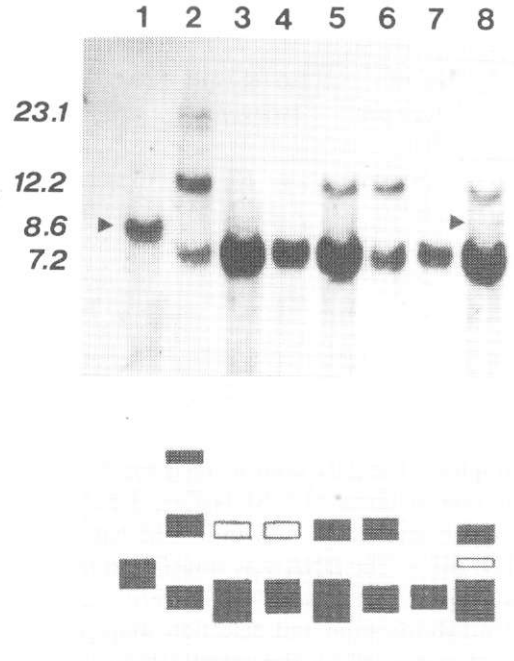

Figure 1: Total DNA of tartary and common buckwheat (lanes 1 and 2, respectively) and putative fusion products (lanes 3-8) probed with ribosomal RNA genes from *Vicia faba*. The arrowheads mark a fragment specific to tartary buckwheat.

Post-fusion protoplasts culture: Cell wall formation was completed after two days and the first cell division occurred after 5-7 days, similar to culture of unfused hypocotyl protoplasts. Mesophyll protoplasts could not tolerate the fusion treatment and after 2-4 days almost all were dead. Even the unfused mesophyll protoplasts in the control dishes did not undergo cell division and the cultures collapsed. However, both fused and unfused hypocotyl protoplasts (control) divided and formed colonies.

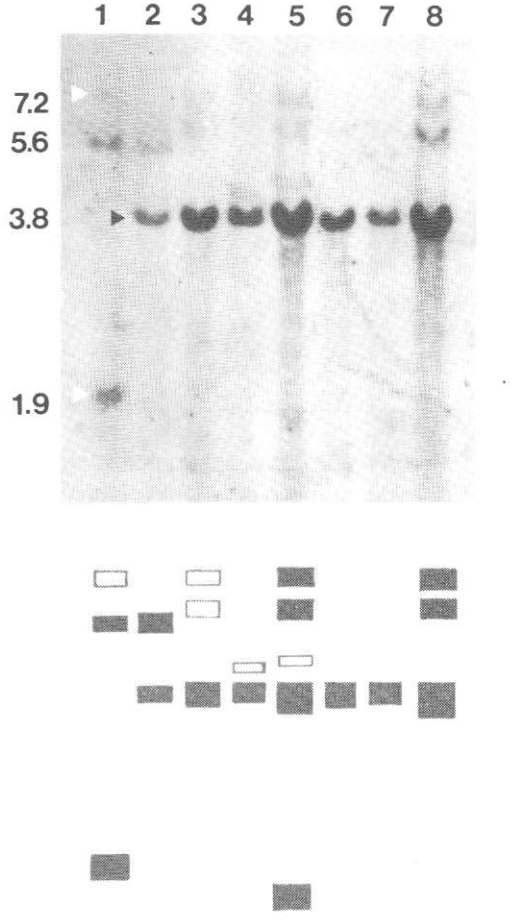

Figure 2: Total DNA of tartary and common buckwheat and putative fusion products probed with the ATP gene of *Beta vulgaris*. Lanes correspond to Figure 1. White arrowheads mark fragments specific for tartary buckwheat. The black arrowhead marks a common buckwheat-specific fragment.

Out of 172 recovered calli obtained in Experiment 2 (Table 3), 26 calli were tentatively identified as calli of *F. tataricum*, according to their visual appearance, i.e. 65% were presumed putative fusion products. *F. tataricum* calli were usually brownish, with a typical shining, silvery plaque on the surface, which

has also been reported by Rumyantseva et al. (1989). Higher numbers of *F. tataricum* calli were observed among the calli transferred at later time periods to CPM or a regeneration medium. This could indicate a growth advantage of fused cells.

Calli cultured for some time (17-60 days) on 2,4-D containing medium developed embryo-like i.e. dome-like structures with a smooth surface two months after protoplast isolation. Unfortunately, they did not evolve into plantlets, but instead developed into calli. Calli without or with only a short 2,4-D treatment (CM5D) did not show any differentiation after four months on regeneration media.

Identification of somatic hybrid calli: The results of the DNA analysis of nuclear, mitochondrial and chloroplast DNA are summarized in Table 4. Although this part of our report was not intended to be a major part of our study, we think it provided some interesting results. The callus in lane 8, for example, could be the result of a fusion event. This particular hybridization pattern displayed a faint band of *F. tataricum* in addition to the major bands of *F. esculentum*. However, most of the calli randomly chosen from among a preselected population of putative fusion products showed patterns rather similar to *F. esculentum* (Figure 1).

The EcoRI digests of both species and the putative fusion products was also probed with a mitochondria gene probe from sugar beet (Figure 2). The callus in lane 8 was again found to possess fragments specific to both parents, similar to the nuclear DNA analysis. Also the pattern in lane 5 showed evidence of a fusion event. In particular, it showed a band at a position characteristic for *F. tataricum*.

A non-random chloroplast segregation was found when probed with the *rbcL* gene from rice chloroplasts (Figure 3), with all the calli analysed showing only the fragment characteristic of *F. esculentum*.

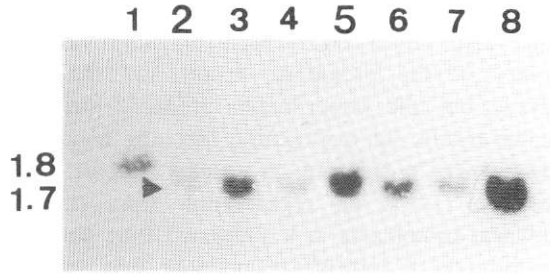

Figure 3: Total DNA of tartary and common buckwheat and putative fusion products probed with the *rbcL* chloroplast gene of rice. Lanes correspond to Figure 1. The arrowhead marks a fragment specific to common buckwheat.

Table 4: Results from DNA analysis of putative fusion products in buckwheat. Total DNA was digested with EcoRI and probed with ribosomal RNA genes (18S and 25S) from *Vicia faba*, the ATP a gene (1.8kb encoding region) of *Beta vulgaris* and the *rbcL* gene (500bp encoding region) from rice.

Callus	Lane	Nuclear genotype	Mitochondria genotype	Chloroplast genotype	Type of fusion product
1	3	E	E+T+R	E	C (H)
2	4	E	E+R	E	(C)
3	5	E	E+T+R	E	C
4	6	E	E	E	U
5	7	E	E	E	U
6	8	E+T	E+T+R	E	H

E = *F. esculentum* T = *F. tataricum* R = Novel bands U = unfused protoplast C = Cybrid H = Hybrid

Discussion

It was possible to achieve cell division after treating protoplasts of two buckwheat species with fusion inducing solutions. However, the results varied widely between the experiments. We were also able to obtain calli as a by-product of our fusion experiments, which were mainly aimed at establishing a fusion technique. A preliminary RFLP analysis of the calli obtained showed some evidence of fusion events. Especially the detection of nuclear rDNA and mtDNA fragments specific to either parent in lane 8 (Figures 1, 2) very strongly indicated a fusion product. However, analysis with only one probe might not be sufficient. Fusion products should be analyzed with various probes as different probes identify different parental parts of fused genoms (Wachocki et al. 1991, O'Connell and Hanson 1985).

Our simple two-step preselection system proved useful for enriching the callus population for putative

fusion products. 1. Use of mesophyll protoplasts as one fusion partner since they did not survive the fusion procedure, as suggested by Menczel and Wolfe (1984). This allowed a 50% selection for fused and unfused cells of the other partner. 2. Hypocotyl protoplasts of *F. tataricum* used as the hauler, as suggested by Adachi et al. (1989), because calli derived from unfused *F. tataricum* protoplasts could later be identified by their appearance.

Although plant regeneration from protoplasts is still an obstacle in buckwheat research, protoplast fusion and culture of fusion products could be greatly improved. There were certainly pitfalls in our experiments, particularly in the RFLP analysis, and further steps must be focused on improving the accuracy of the protoplast fusion and the DNA analysis, but the experiments produced first and valuable results for overcoming breeding barriers in buckwheat. Considering a recent report from Gumerova (1991) on regeneration from common buckwheat protoplasts in a shortened time period, a breakthrough in buckwheat breeding may soon be expected.

Acknowledgements

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Fagopyrum 14 (1994): 13 - 16

Obtaining of interspecific buckwheat hybrid (*Fagopyrum esculentum* Moench x *Fagopyrum cymosum* Meissn.)

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Key words: *F. esculentum*, *F. cymosum*, hybrid, ovule culture

Abstract

As a result of crossing of two buckwheat species, *F. esculentum* and *F. cymosum*, using ovule culture, hybrid plants were obtained. Interspecific hybrids possessed a phenotype close to *F. cymosum* and were characterized by complete sterility in all pollination types.

Medvrstni križanec ajde (*Fagopyrum esculentum* Moench x *Fagopyrum cymosum* Meissn.)

S pomočjo uporabe kultur ovul so avtorji dobili medvrstnega križanca *Fagopyrum esculentum* in *F. cymosum*. Vsi tipi cvetov so bili sterilni, fenotip križancev je bil podoben *F. cymosum*. (Prevod uredništva).

Introduction

The genus *Fagopyrum*, according to Krotov's (1) classification, includes the following species: *F. esculentum* Moench, *F. tataricum* (Z) Gaerth., *F. cymosum* Meissn., *F. suffruticosum* Fr. Schmidt, *F. ciliatum* Jaeg. f., and *F. giganteum* Krotov. Several wild buckwheat species have been discovered in China (2). *F. giganteum* is an amphiploid, released by Krotov through hybridization of tartary and cymose buckwheat (3). Obtaining interspecific hybrids with *F. esculentum* through conventional methods has not been successful.

In cross combination of *F. esculentum* with *F. tataricum*, with *F. esculentum* as the maternal form, progamic incompatibility takes place, revealing suppression of pollen tube growth of *F. tataricum* in the pistil tissue of *F. esculentum*. In reverse cross combination, the pollen tubes grew in some cases up to the pistil base of *F. tataricum*, though hybrid embryos died several days after fertilization (4, 5, 6).

In crossing *F. cymosum* with *F. esculentum*, the pollen tubes of *F. esculentum* grew through up to a certain portion of the *F. cymosum* style, but fertilization did not occur (7). When using long-styled plants of *F. cymosum* as the maternal form, fruit-setting occurred but in quite a few cases, the ovaries subsequently died off (6). In reciprocal combination with *F. esculentum* as maternal form, the pollen tubes grew normally, though postgamic incompatibility was evident (6, 7).

To overcome incompatibility of interspecific crossing, a method of isolated embryo culture was used. The first attempts with hybrid embryos culture in vitro were not successful. Embryos in artificial medium did not grow (4, 6).

In cross combination of *F. esculentum* with *F. giganteum*, a callus was obtained from the hybrid embryo (8). Experiments were carried out in growing and cloning in vitro 10-12 days old embryos of different crossing combinations, but the authors are not sure if all the embryos were hybrids (9).

The first description of interspecific buckwheat hybrids with a combination of *F. esculentum* x *F. cymosum* was performed by Ujihara, Nakamura and Minami (10). They used ovule culture.

We started research in obtaining interspecific hybrids in 1987. This paper presents the results of in vitro cultivation of buckwheat hybrid ovules of combination *F. esculentum* x *F. cymosum* and characterizes the hybrid plants obtained.

Material and Methods

Parents were two buckwheat species: *F. esculentum* and *F. cymosum*. Plants of both species were raised in a greenhouse. Crossings were done in autumn or spring 1987-1991. The maternal form in all cases was *F. esculentum*, whose flowers were pollinated with *F. cymosum* pollen. Short-styled flowers were emasculated before pollination before anther dehiscence; long-styled flowers were pollinated

without sterilization. Stigma cleanliness before hybridization and pollen presence after pollination was confirmed with a magnifying glass. Ovules with developing embryos were isolated from 4-6 days ovaries and cultured in vitro according to a special method elaborated for immature buckwheat embryos of *F. esculentum* (11, 12).

Results and Discussion.

When *F. esculentum* was pollinated with *F. cymosum* pollen, about half of the flowers set ovaries which later died off. Death of ovary walls was preceeded by death of ovules. Ovules not older than 6 days are considered best fit for in vitro culture; more matured ovules became necrotic in the medium.

In our experiment, we induced four replications of crossings, and pollinated 615 flowers in total, and planted 182 ovules onto the medium (Table 1). Hybridization effectiveness varied from 18 to 58 percent.

The majority of cultured ovules died off either because of infection or degeneration of hybrid embryos. In all, 29 embryos (16 percent of explants) continued their development in vitro. Many of them formed callus tissue but formed abnormal seedlings.

Calli developing from hybrid embryos were of various morphology with different growth rate and living period. Some embryos formed gray or pale-yellow calli, not big in size. This type of callus developed slowly and died off in 1-2 subcultures. Another group of calli, obtained from hybrid embryos, was characterized by dense consistence and high growth rate. Callus colouring varied from yellow to green and rose-red. It was notable that callus colouring and structure could transform in the course of cultivation within a single callus clone.

Some callus clones survived in vitro culture for several years. As time passed, the growth intensity of callus tissue subsided, the structure became friable, the colour changed into gray-yellow, necrotic spots appeared and the calli died off.

Attempts to obtain regenerants from long-passaged hybrid calli were not successful. The application of various growth regulators resulted in induction of green areas with dense globular structure, but shoots did not emerge.

Only one of the six-day old embryo calli of the 1987 hybridization possessed regeneration potential. In the course of the initial months of cultivation, buds emerged and morphogenic tissue with high shoot-forming potential was obtained.

The first hybrid shoots were transferred into soil 7 months after the ovule was planted on medium. Thus we obtained the first hybrid clone, which has been maintained in culture since 1987 (Figure 1). The shoot forming capability was maximum during the first two years of cultivation; later, the rate of new buds and shoot formation subsided. Shoots obtained during subcultivation were transferred into soil (Figure 2).

Embryos could develop without callus formation, but since the embryo was premature, deviations from normal development were observed; abnormal plantlets with different morphologic structures of submature roots and cotyledons or abnormal shoots were formed.

Such plantlets developed differently in the course of subsequent subcultivation. Some of them died off after 1-2 subcultures, some lived a bit longer but

Table 1: Results of in vitro culture of buckwheat hybrid ovules.

Date of hybridization	Crossing combination	No. of pollinated flowers	No. of planted ovules	Infected	Degenerated	Developing
Autumn	L x S	58	25	16	8	1 shoot
1987	S x L	130	55	22	28	5 calli
Spring	L x S	31	18	8	9	1 callus
1988	S x L	33	12	1	5	6 calli
Spring 1989	S x L	123	28	3	13	10 calli 2 shoots
Spring 1991	S x L	240	44	19	21	1 callus 3 shoots

S - short-styled plants

L - long- styled plants

failed to form vital shoots. We managed to preserve two hybrid clones, one from the 1989 hybridization (4 days old embryo) and one from 1991 (5 days old embryo). The embryos which initiated these clones formed morphogenic tissue with numerous shoots in 1-2 months. In 3 months, the first hybrid shoots were planted into soil.

Thus, at present, three hybrid clones are maintained in culture in vitro which constantly give shoots. Shoots transferred into pots with soil have developed into plants.

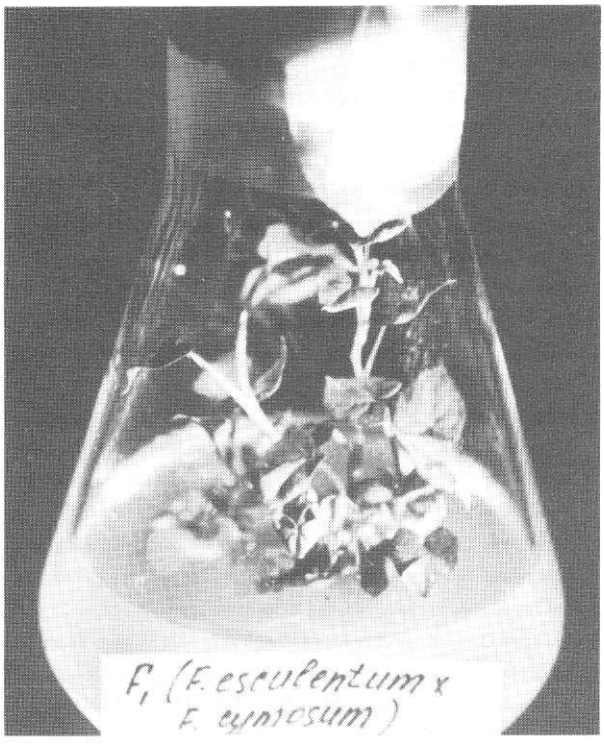

Figure 1: Hybrid clone in in vitro culture.

Hybrid shoots and plants have morphobiological peculiarities which differ from the parent species. The most striking differences are observed between hybrids and maternal species *F. esculentum*.

Differences are also found among hybrid clones. Hybrid clones of 1987 and 1991 morphologically most resembled each other. Shoots of these clones easily survived after being transferred into non-sterile conditions. There was 100 percent hybrid survival, while survival of regenerative shoots of *F. esculentum* barely reached 50 percent. The hybrid clone of 1989 differs from the other two in it's total inability to survive in vivo. Under in vitro cultivation the shoots of this clone were identical to the others; on being transferred onto rhizogenic medium they produced numerous roots, similar to the others. But outside of the culture it was impossible to preserve even a single plant. In soil, all shoots instantly died without continuing their development.

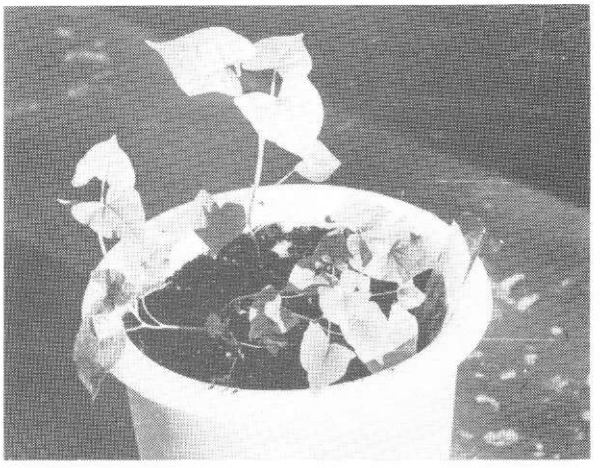

Figure 2: Hybrid shoots transferred into soil.

The distinctive feature of hybrids is their high sensitivity to the spectral composition of light. Natural light, coming through glass, provides optimal development of plants. In conditions of artificial illumination, where both parent species show normal development, hybrids show considerable shortening of internodes.

Normally developed hybrid plants of clones from 1987 and 1991 phenotypically more resembled the paternal species *F. cymosum* and were characterized by intensive branching (Figure 3). Hybrid plants from 1987 were long-styled, while plant flowers from the 1991 hybridization possessed long stamens and long pistils, with the pistil longer than the stamens. After pollination of hybrids with pollen of maternal and paternal species, as well as with no pollination at all, some plants set ovaries which later died off. Seeds from hybrid plants were not obtained.

Conclusions

1. Interspecific hybrids were obtained in crossing combinations of *F. esculentum* x *F. cymosum* with the application of ovule culture.
2. Hybrid plants possessed morphological traits similar to the paternal species, *F. cymosum*, and were characterized by complete sterility.

Acknowledgement

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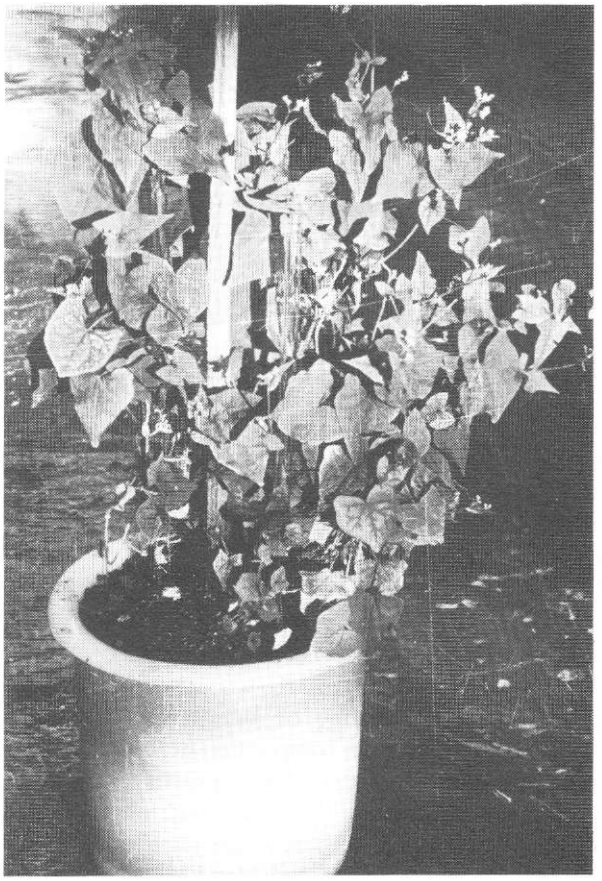

Figure 3: Hybrid plants *F. esculentum* x *F. cymosum*.

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Buckwheat in Karakoram and the Hindukush*

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Key words: Jawas (common buckwheat), braw (tatary buckwheat), weedy form of tatary, allozyme variability, buckwheat diffusion in Pakistan

Abstract

Both common and tatary buckwheat are cultivated in the valleys of the Indus river and its tributary, the Shyok river in Ghanche and Baltistan, and also in Hunza and Nagar. Common buckwheat is called "jawas", while tatary buckwheat is called "braw (or brow)". Wild tatary buckwheat, *F. tataricum* ssp. *Potanini* is also grown in farmers' fields throughout northern Pakistan. In addition, the third type of tatary buckwheat, the weedy form, also grows in the Hushe valley of Ghanche and the Indus valley of Baltistan. It morphologically resembles cultivated tatary, but it has the characteristics of wild species, a shattering habit and strong dormancy. The greatly expected drastic change of gene frequency was not observed in the allozyme variability of common buckwheat in northern Pakistan, probably due to its large scale cultivation in a well suited rotation of crops in this region. A unique variant at the Pgm-1 locus in tatary buckwheat was detected in cultivated populations in the Hushe valley. Based on allozyme variability and distribution of buckwheat species, the diffusion processes in this region are discussed.

Ajda v Karakoramu in Hindukušu

V porečju reke Ind, zlasti ob reki Šiok,v Ganči in Baltistanu, ter v Hunzi in Nagarju, uspevata navadna in tatarska ajda. Navadno ajdo imenujejo "javas", tatarsko pa "brav" ali "brov". Na kmečkih poljih v severnem Pakistanu raste divja tarska ajda *F. tataricum* ssp. *Potanini*. Poleg tega raste še tretja oblika tatarske ajde, kot plevel, in sicer v dolinah Huše in Inda. Po obliki je podobna gojeni tatarski ajdi, toda ima tudi lastnosti divje vrste, saj se osipa in ima izrazito dormantnost semen. Glede alocimske raznolikosti navadne ajde, ni bilo opaziti sicer pričakovane spremembe pogostnosti genov, verjetno zaradi širokega pridelovanja ob dobrem povpraševanju po pridelku. Ugotovljena je bila posebna oblika na Pgm-1 lokusu pri gojeni populaciji tatarske ajde v dolini Huše. Na osnovi alocimske variabilnosti in razporeditve vrst ajde avtor razpravlja o širjenju ajd v tem območju. (Prevod uredništva).

Introduction

Our knowledge of the cultivation and utilization of buckwheat in Pakistan is very scarce. Even the IBPGR workshop on buckwheat held at Tsukuba in 1991 described nothing on this issue (IBPGR 1992). On the other hand, my previous research on the diffusion of buckwheat cultivation in the Himalayan regions (Ohnishi 1985, 1993a, Ohnishi and Nishimoto 1988) suggested that if buckwheat is cultivated in northern Pakistan, it is the western terminus of diffusion of buckwheat cultivation. Fixation, elimination and drastic changes of allele frequency at isozyme loci were therefore expected, as actually found in Kumaun, Garhwal and Kashmir of India (see Ohnishi 1993c for more details).

As for wild buckwheat species, perennial buckwheat, *F. cymosum* Meisn. (*F. dibotrys* (D. Don) Hara), has been reported from Afganistan (Kitamura 1960), so there was a possibility of seeing it in northern Pakistan. Since wild tatary buckwheat, *F. tataricum* ssp. *Potanini* Batalin, is widely distributed throughout Sichuan and Tibet of China, it is highly possible for the species to have spread to northern Pakistan through old trading routes.

In this article, I will report what I found on the distribution and cultivation of buckwheat species during a six week journey in Karakoram and the Hindukush in the fall of 1993. In addition, the results of allozyme analyses by starch gel electrophoresis on cultivated buckwheat, both common and tatary, will also be reported. They will provide a clue to understanding the diffusion of buckwheat in this area.

Cultivation of buckwheat in Karakoram and Hindukush

My travel route is briefly sketched on the map in Figure 1. The Northern area includes the former Gilgit agency, Baltistan and Ghanche districts, but it

* Contribution from the Plant Germ-Plasm Institute, Fac. of Agriculture, Kyoto Univ. No. 66.

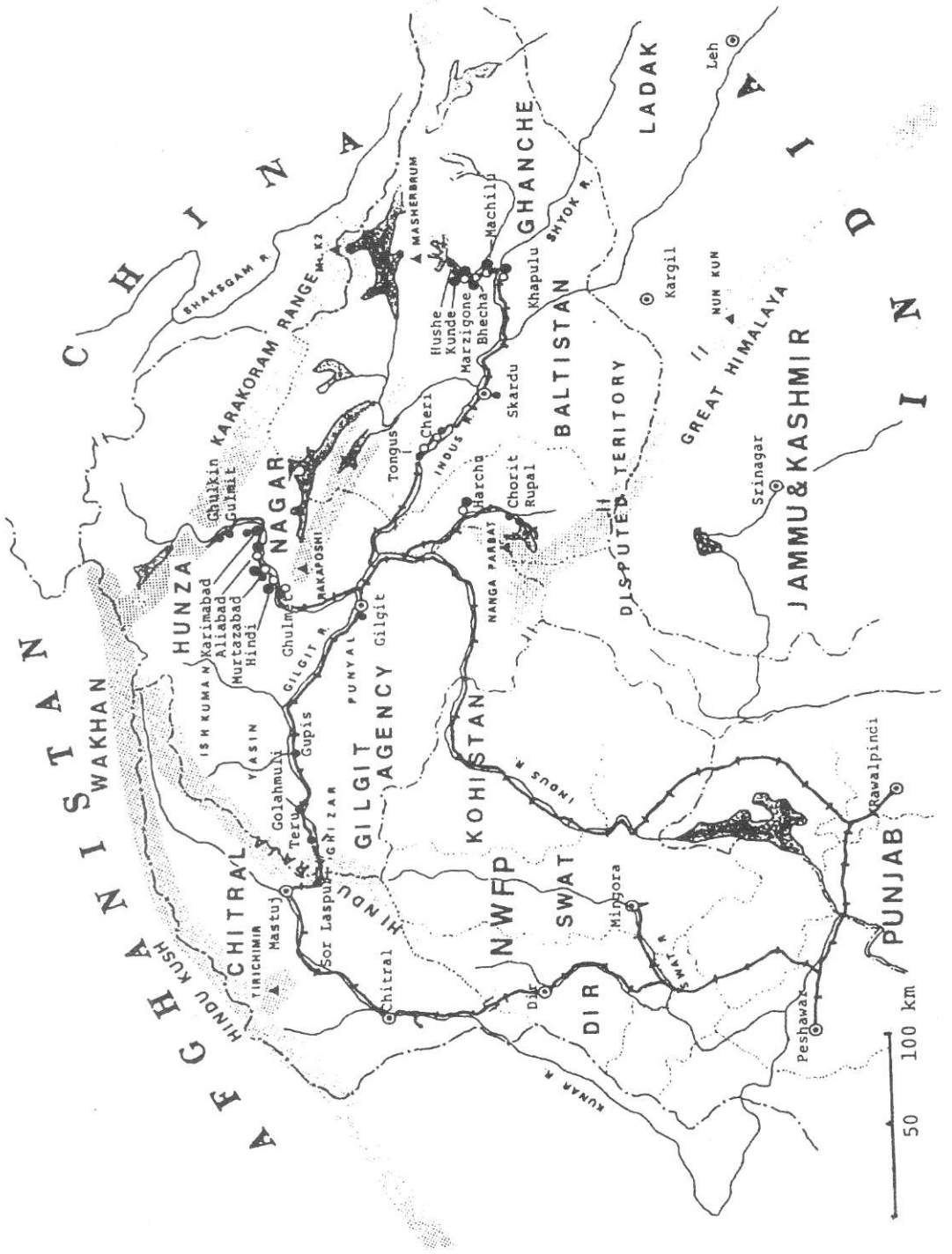

Figure 1: My travel route and the distribution of buckwheat species in northern Pakistan.

- ● *F. tataricum* (cultivated form)
- ○ *F. esculentum*
- • *F. tataricum* ssp. *Potanini* (wild and weedy forms)
- ⊙ Major city town
- +++ Travel route

is not a proper province of Pakistan. It is, rather, the area formerly governed by the Maharaja of Kashmir and now the disputed area of Kashmir controlled by the Pakistan government. Chitral is separated from the Northern area by Hindu Raja to the east and bounded from Afganistan (to the west) by the Hindukush range (see Figure 1). Travelling through the Northern area and NWFP is entirely free even for foreigners, although travellers to a number of specific village districts are required to have permission from the police station.

General features of agriculture

Agricultural activity in Karakoram and Hindukush is limited to the valleys of the Indus river, the Kunar river and their tributaries. The soil in the valleys is formed by alluvial deposits from mountains and streams. The land in the valleys is cultivated wherever the supply of water is enough for crops and the configuration of the ground is suitable. The water supply depends on artificial irrigation; in most cases, water from tributary streams (locally called Gol) is used, but occasionally, the water of glaciers is used directly for irrigation. In most of these valleys, the climate allows two crops a year. Staple crops are wheat and barley, both are winter crops, and they are consumed as chapatti or nan, which is the major food throughout the country. In warmer lower valleys such as Chitral and Swat, rice and maize become most important, while in higher elevated regions, only one crop is possible a year, and barley and wheat are mainly cultivated from fall to the next summer. Buckwheat and millets are cultivated as a summer crop after the winter crop in the belt just below the one-crop zone, where the summer crop period is very short and maize cannot be grown.

Millets cultivated in this region include fox tail millet, *Setaria italica* Beauv., millet, *Panicum miliaceum* L. and sorghum, *Sorgum bicolor* Moench, but not finger millet, *Eleusine indica* Gaertn. This shows a sharp contrast with millet cultivation in the Nepal and Indian Himalayas, where finger millet predominates.

Buckwheat species in northern Pakistan

Both common and tatary buckwheat are cultivated in Karakoram and Hindukush as a summer crop (see Figure 2). In the Indus valley in Baltistan, common buckwheat is utilized as buckwheat groats, while tatary buckwheat is milled and used to make chapatti or nan. Wild tatary buckwheat also grows, although it is morphologically slightly different from that found in Sichuan and Tibet of China (compare Figures 3B, 3D). It is characterized by very low height (40 cm or less) and quite a short growing period (32 days from sowing to flowering, as compared with 37 days for wild tatary in Sichuan and 39 days for cultivated tatary in southern China). It usually grows in farmer's fields, among barley, wheat, maize, potato and buckwheat and is rarely found on roadsides or mountain slopes. In addition, a weedy form of tatary also grows in the Hushe valley of Ghanche district and the Indus valley of Baltistan. It morphologically resembles cultivated tatary but has the characteristics of wild species, a shattering habit, strong dormancy and much branching (see Figure 3C).

The villages at which I could sample cultivated or wild tatary buckwheat are plotted on the map in Figure 1.

In the Indus valley in Ghanche and Baltistan, i.e. Khapulu, Skardu, Cheri and Tongus in Figure 1, common buckwheat is called "jawas", while tatary buckwheat is called "braw (or brow)", although the languages used in northern Pakistan are complicated and quite different from locality to locality (see Biddulph 1880 for languages in northern Pakistan). This is true along the Indus valley and its tributaries up to Gilgit. In contrast, in the villages in Hunza and Nagar (see Figure 1) tatary and common buckwheat are not distinguished by usage (both mainly for chapatti) or name; "braw (or brow)" is the name for both common and tatary buckwheat (except in the village Hindi, often mentioned as Hini but recently more frequently called by its Pakistan name, Narisabad, where tatary buckwheat is called "ghaqau", and "brow (or Boli)" is used for common buckwheat as opposed to tatary buckwheat. In the upper Hunza (i.e. Gojal) "Boli" is commonly used for buckwheat (both common and tatary) and wild tatary growing here is also called by this name.

In the upper valleys of the Gilgit river, that is, Yasin, Ishkuman and Ghizar (see Figure 1) buckwheat cultivation is rare, but wild tatary commonly grows in wheat or maize fields. Buckwheat is called "braw (or brow)" in this region. The distribution of wild tatary buckwheat seems to extend westward, crossing Hindu Raja, up to Sor Laspur (see Figure 1), but I failed to find it in Harchin, just 5 km north of Sor Laspur, in spite of searching for it for four hours in wheat, barley, tobacco and hemp fields. My search for this species in Chitral and Swat valleys also resulted in complete failure. I was unable, too, to see *F. cymosum* in Karakoram and Hindukush; this species is a weed commonly found in Nepal, Kumaun and Garhwal, as well as the Indian part of Kashmir (Gohil et al. 1983, Ohnishi 1993a). The climate,

particularly the arid soils in Karakoram and Hindukush, does not allow *F. cymosum's* distribution in this area.

Allozyme variability in tatary buckwheat

22 loci affecting 15 enzymes were assayed for allozyme variability for 5-10 individuals in each of 24 Pakistan populations by using a standard starch gel electrophoresis (see Ohnishi and Nishimoto 1988 for procedures). Tatary buckwheat has only a few variants as a species; most of the variants are found in wild populations in Sichuan province of China and almost all cultivated land races from various parts of the world and wild populations from Tibet have the same common allele at all loci (Ohnishi 1993a). The cultivated and wild (both true wild and weedy forms) populations in Karakoram and the Hindukush had the same common allele at each locus. The slow allele found at the Pgm-1 locus is the only variant in Pakistan. This allele has never been reported from anywhere else in the world. The cultivated populations of Machilu and Kunde, both located in the Hushe valley of Ghanche district (Figure 1) shared the variant allele. The mutation probably arose after tatary's arrival in this valley.

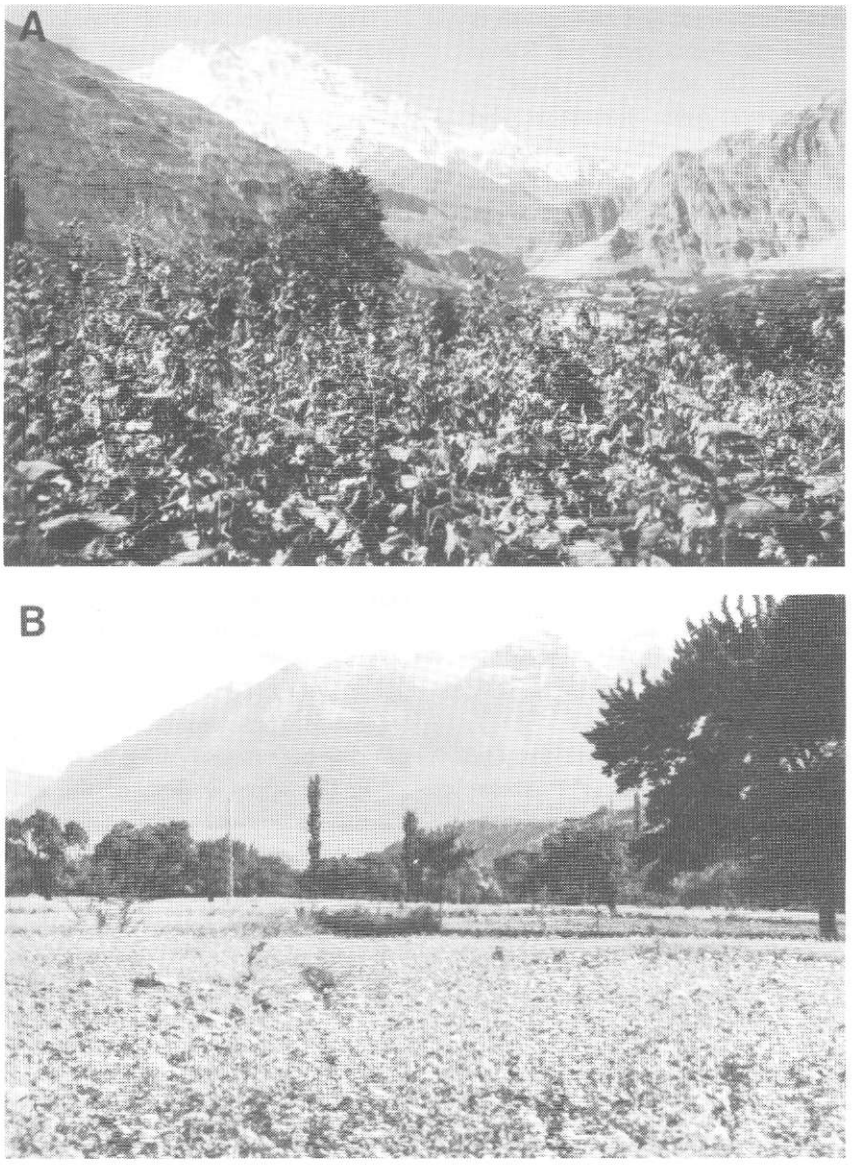

Figure 2: Cultivation of buckwheat in northern Pakistan: (A) cultivation of tatary buckwheat in Hunza. The high mountain in the background is Rakaposhi (7788 m), (B) common buckwheat fields surrounded by apricot orchards in the Hushe valley in Ghanche district.

Allozyme variability in common buckwheat

Eleven populations of common buckwheat in Karakoram and Hindukush were assayed routinely for its 19 loci affecting 12 enzymes, using the standard method of starch gel electrophoresis (see Ohnishi and Nishimoto 1988 for procedures, the enzymes and the loci studied). The allele frequency at ten polymorphic loci in each population is shown in Table 1, where additional data on the allele frequency for western Nepal, Indian Kashmir, Tibet and the silk road are provided for comparisons.

The highly expected drastic change of gene frequency was not observed at any polymorphic locus in Pakistan populations. The average heterozygosity ranges 0.125-0.136, which is slightly less than the average of Chinese or Nepali populations (about 0.15) but slightly higher than the Kashmiri populations (0.10). The percentage of polymorphic loci is 36.8-47.4%, which lies between the values of the Nepali and the Kashmiri populations.

Most of the Pakistan populations have lost the variability at 6-Pgdh-1, Adh and Gdh loci, but still maintain the F allele at the Pgm-2 locus; this allele has been completely lost from Kashmiri populations.

The genetic distance (Nei 1972) between all pairs of populations in northern Pakistan and between pairs of a Pakistan population and another adjacent region is given in Table 2. It is apparent that the distance among the Pakistan populations is at most 0.010, usually between 0.001-0.007. Pakistan populations, as a group, have the shortest distance to Kashmir (on average 0.0079) and relatively short distance to the silk road (0.081) and the longest distance to western Nepal (0.0140). This result does not immediately lead to an understanding of the diffusion processes of common buckwheat in Karakoram and Hindukush; the issue will be discussed later.

Discussion

In northern Pakistan, three distinct groups of tatary buckwheat are cultivated or grow in the wild; cultivated, wild and weedy forms of tatary buckwheat. From the distribution of *Fagopyrum* species in the great Himalayas and Tibet, I (Ohnishi 1993a) concluded that wild tatary *F. tataricum* ssp. *Potanini* diffused from its original birth place, Sichuan province of China, to eastern Tibet, then to the Yalutsangpu river valley in Tibet, while cultivated tatary mainly diffused along the southern hills of the Himalayas. If this is true, the wild tatary buckwheat in Karakoram and Hindukush must come from Tibet, probably via Ladak through the old trading routes (Figure 1).

The weedy tatary with wild characters such as a shattering habit and strong dormancy, yet morphologically similar to cultivated tatary buckwheat, has never been reported from anywhere else in the world, except from the Indian part of Kashmir, where I found it among tatary buckwheat samples in 1986. Taking diffusion processes in the Himalayas into account, I arrive at an hypothesis on the origin of this weedy tatary buckwheat. At an early stage of diffusion, wild and cultivated tatary took distinct routes of diffusion: the wild one took the route into Tibet and the cultivated one took the south Himalayan route. When the two tatary arrived at Kashmir or Karakoram, they reunited. The two types were hybridised and the weedy type was born out of the hybridization. Future experimental or expeditionary observations will test the hypothesis.

Fairly common and large-scale cultivation of both common and tatary buckwheat in the valleys of the Indus river and its tributary, the Shyok river in Baltistan and Ghance districts and in Hunza and Nagar (Figure 1) is first reported in this article. They are cultivated as a summer crop after a winter crop, either barley or wheat. Common buckwheat in the Indus valley of Baltistan has white flowers, in contrast with pink flowers in Hunza and Nagar and in the Hushe valley of Ghanche district. White flowered populations have a better yield. As discussed in Ohnishi (1993b), pink flowered varieties are probably primitive as compared with white ones, although Chai et al. (1992) came to an opposite opinion. In any case, at least two waves of diffusion of common buckwheat have taken place here in northern Pakistan; first pink flowered varieties and later white ones.

Allozyme variability shown in Table 1 provides no further elucidation of this. However, as I discussed in Ohnishi (1993a) cultivation of common buckwheat mainly diffused along the southern slopes of the Himalayas. It entered Nepal from the east, then spread to Kumaun and Garhwal of India; while Tibetan common buckwheat came relatively recently from Sichuan province of China (Ohnishi 1993a). As shown in Table 2, the genetic distance between northern Pakistan and Kashmir is the shortest, as we expected. A relatively long distance between Pakistan and Tibet implies no connection between them in common buckwheat, although wild tatary in Karakoram probably came from Tibet, as discussed earlier. Some rather unexpected results are: (1) Pakistan populations have a relatively short distance to the silk road. (2) Drastic changes of gene frequency such as observed in Kumaun, Garhwal and Kashmir were not observed in Karakoram.

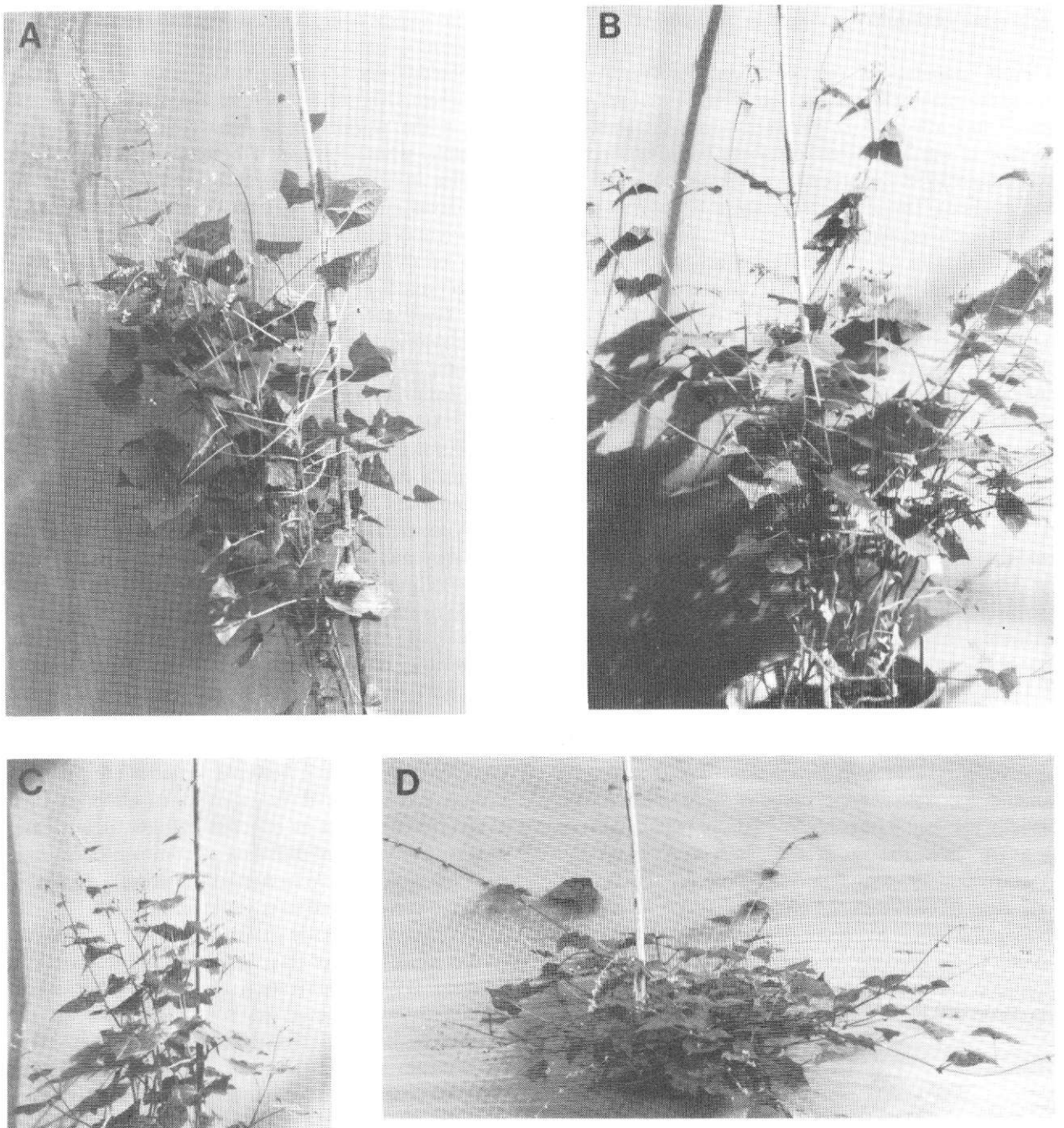

Figure 3: Four distinct forms of tatary buckwheat.
A: Cultivated tatary buckwheat from Aliabad, Hunza.
B: Wild tatary buckwheat from Markan, Sichuan province of China.
C: The weedy form of tatary buckwheat from Tongus in the Indus valley of Baltistan.
D: Wild tatary buckwheat from Gilgit.

Table 1: Allele frequencies at polymorphic loci.

Population	6-Pgdh-1			Pgm-1			Pgm-2			Mdh-1		Mdh-3				Adh		
	S	N	F	S	N	F	S	N	F	F	N	U	S	N	F	S	N	F
1. Aliabad	0.8	98.9	0.3	20.8	79.2	0.0	5.0	92.0	3.0	35.0	65.0	0.0	15.0	70.0	15.0	0.0	99.0	1.0
2. Murtazabad	0.5	99.5	0.0	21.8	78.2	0.0	3.5	94.7	1.8	32.0	68.0	0.0	10.2	72.0	17.8	0.5	99.0	0.5
3. Hindi	0.2	99.8	0.0	15.5	84.5	0.0	6.0	87.0	7.0	40.5	59.5	0.0	14.8	70.4	14.8	0.3	99.2	0.5
4. Ghulmet	0.0	100.0	0.0	15.5	84.5	0.0	7.5	87.0	5.5	22.5	77.5	0.0	9.5	76.0	14.5	0.3	99.7	0.0
5. Harchu	0.0	100.0	0.0	15.5	84.5	0.0	7.3	88.9	2.8	24.5	75.5	0.0	14.0	71.5	14.5	0.3	99.7	0.0
6. Tongus	0.0	100.0	0.0	19.5	80.5	0.0	5.5	91.7	2.8	33.0	67.0	0.0	14.8	70.3	14.9	0.0	100.0	0.0
7. Cheri	0.5	99.2	0.3	14.3	85.7	0.0	5.3	90.2	4.5	22.5	77.5	0.0	11.8	69.7	18.5	0.0	100.0	0.0
8. Khapulu	0.8	99.2	0.0	14.2	85.8	0.0	7.5	84.0	8.5	28.5	71.5	0.0	11.3	80.3	8.4	0.0	100.0	0.0
9. Machilu	1.0	99.0	0.0	20.3	79.7	0.0	8.0	86.7	5.3	26.0	74.0	0.0	12.5	70.7	16.8	1.3	98.7	0.0
10. Bhecha	0.5	99.5	0.0	18.3	81.7	0.0	7.0	90.7	2.3	27.0	73.0	0.0	12.0	71.5	16.5	0.5	99.5	0.0
11. Marzigone	0.3	99.7	0.0	19.8	80.2	0.0	5.0	91.2	3.8	25.8	74.2	0.0	11.0	72.2	16.8	0.5	99.5	0.0
12. Silk road (6)*	6.1	93.7	0.3	11.3	88.4	0.3	14.5	80.6	4.9	38.6	61.4	0.1	20.9	62.1	16.9	0.2	99.3	0.5
13. Kashmir (2)	0.1	99.9	0.0	9.7	90.3	0.0	8.5	91.5	0.0	28.0	72.0	0.0	4.6	83.1	12.3	0.0	100.0	0.0
14. Tibet (4)	4.9	95.0	0.1	6.3	93.7	0.0	6.6	88.7	4.7	51.0	49.0	0.8	17.6	68.8	12.8	0.0	99.1	0.9
15. West Nepal (5)	1.4	98.5	0.1	9.4	90.3	0.3	16.2	73.4	10.4	46.4	53.6	0.1	21.2	64.7	14.0	4.5	95.1	0.4

*: average of the number of populations shown in parentheses.

Population	Got-2			Dia-2			Sdh-1			Gdh			% of polymorphic loci	Average heterozygosity
	U	S	N	S	N	F	U	S	F	S	N	F		
1. Aliabad	6.0	55.8	38.2	4.5	92.9	2.6	0.0	62.7	37.3	0.0	100.0	0.0	47.4	0.136
2. Murtazabad	4.5	64.8	30.7	8.6	89.8	1.6	0.0	59.2	40.8	0.0	99.7	0.3	42.1	0.132
3. Hindi	5.3	61.4	33.3	6.8	92.1	1.1	0.0	69.2	30.8	0.0	99.7	0.3	36.8	0.134
4. Ghulmet	6.0	51.2	42.8	8.9	90.3	0.8	0.3	71.2	28.5	0.0	100.0	0.0	36.8	0.125
5. Harchu	2.0	60.0	38.0	6.5	91.9	1.6	0.5	67.2	32.3	0.0	100.0	0.0	36.8	0.125
6. Tongus	3.3	65.2	31.5	7.0	91.9	1.1	1.0	61.5	37.5	0.0	100.0	0.0	36.8	0.130
7. Cheri	3.8	56.2	40.0	5.9	91.9	2.2	0.0	50.0	50.0	0.0	100.0	0.0	36.8	0.128
8. Khapulu	5.3	67.3	27.4	7.2	92.8	0.0	0.0	65.4	34.6	4.3	95.4	0.3	42.1	0.128
9. Machilu	5.5	70.2	24.3	7.1	91.2	1.7	0.0	62.0	38.0	0.5	99.5	0.0	47.4	0.134
10. Bhecha	5.5	61.5	33.0	6.5	92.5	1.0	0.0	63.5	36.5	0.0	100.0	0.0	36.8	0.128
11. Marzigone	5.5	65.0	29.5	6.6	93.4	0.0	2.0	62.5	35.5	0.8	99.2	0.0	36.8	0.128
12. Silk road	12.9	53.2	33.9	4.8	95.1	0.1	0.2	53.4	46.4	0.0	100.0	0.0	42.1	0.151
13. Kashmir	0.6	75.4	24.0	2.6	97.4	0.0	0.3	49.3	50.4	0.0	100.0	0.0	36.8	0.103
14. Tibet	6.5	53.7	39.8	1.7	98.3	0.0	1.1	56.9	42.0	0.0	100.0	0.0	42.1	0.132
15. West Nepal	9.0	43.4	47.6	6.4	90.6	3.0	0.2	62.9	36.9	0.2	99.3	0.5	47.4	0.156

Table 2: Genetic distance between populations or distinct groups of populations.

	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Aliabad	0.002	0.002	0.005	0.003	0.001	0.005	0.005	0.004	0.002	0.003	0.006	0.010	0.007	0.009
2. Murtazabad		0.004	0.007	0.003	0.001	0.005	0.004	0.002	0.001	0.001	0.008	0.006	0.012	0.016
3. Hindi			0.006	0.004	0.002	0.010	0.004	0.005	0.003	0.004	0.007	0.011	0.006	0.008
4. Ghumet				0.001	0.006	0.007	0.005	0.007	0.003	0.004	0.012	0.014	0.017	0.013
5. Harchu					0.002	0.005	0.003	0.003	0.001	0.001	0.009	0.009	0.013	0.013
6. Tongus						0.005	0.003	0.002	0.001	0.001	0.006	0.006	0.009	0.013
7. Cheri							0.008	0.006	0.004	0.004	0.007	0.007	0.014	0.016
8. Khapulu								0.002	0.003	0.002	0.010	0.006	0.013	0.016
9. Machilu									0.001	0.001	0.009	0.006	0.016	0.019
10. Bhecha										0.000	0.007	0.007	0.012	0.014
11. Marzigone											0.009	0.006	0.014	0.017
12. Silk road												0.012	0.004	0.006
13. Kashmir													0.015	0.024
14. Tibet														0.005
15. West Nepal														

Phylological notes on the names of buckwheat mentioned in the section on results suggest that buckwheat first entered the Indus valley, then went to the Gilgit region, and finally arrived at Hunza and Nagar (Figure 1). In contrast, a short genetic distance between Pakistan and the silk road may suggest that common buckwheat in Pakistan may have come from the north via the silk road. However, this is probably not the case. Historical consideration of transmittance of crops via the silk road by Laufer (1919) asserted that fewer crops than we imagined, only a small number, actually diffused through the silk road. Buckwheat shows no sign of transmittance from China to Afganistan and Persia.

The issue which we should therefore consider seriously is why the differentiation of gene frequency at isozyme loci occurred in Kuman, Garhwal and Kashmir of India, and why not in Karakoram. At present, I have no solid answer to this. I can, however, suggest a possible solution. Cultivation and utilization of buckwheat (probably millets, too) seems to have been adequately taken into the agricultural system in Karakoram. Both common and tatary buckwheat are cultivated as a summer crop in rotation of crops and they are consumed as a major food, chappati. The history of cultivation of buckwheat in this region is not known, but Schomberg (1935) already described common cultivation of buckwheat in Hunza and Nagar, and reported that buckwheat was used for the festival of Tum-i-shilling, with the filling of an animal stomach with buckwheat flour (jokish in Hunza). The cultivation and utilization of buckwheat is large scale and stable in time in northern Pakistan, while in Kumaun and Garhwal, buckwheat is utilized in a peculiar manner; young leaves of common buckwheat are used as a vegetable and buckwheat flour is mainly consumed as paphal, dried thin pancake with curry and sesame seasoning (The word "paphal" means no more than "buckwheat" in Nepal).

From the viewpoint of variability of buckwheat populations, I can say that the population size in Karakoram is larger and more stable than in Kumaun, Garhwal and Kashmir. Hence we may assume that common buckwheat, once introduced into Karakoram from India, did not change very much in the genetic constitution of populations; while in Kumaun, Garhwal and Kashmir, where the population size was small and unstable, drastic changes of gene frequency took place by random drift. This hypothesis does not contradict any experimental or observed data, but it has no solid basis and it cannot explain why the genetic distance between Pakistan and the silk road is so short.

Future experimental or expeditionary observation for buckwheat in Kashmir and Himachal Pradesh of India may clarify the diffusion processes in the western part of the Great Himalayas and Karakoram and the Hindukush.

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Evaluation of buckwheat breeding materials obtained after crossing with dwarf forms

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Key words: dwarf, lodging resistance, stem length

Abstract

Using buckwheat starting material of different stem length, plants of intermediate type, with compact stem and standard height, were obtained. Plants with number 31/78 show good architectonics, those with numbers 457/78 and 404/78 have high ecological plasticity and provide valuable starting material for further breeding.

Ocena uporabnosti križancev z geni za pritlikavo rast za žlahtnjenje ajde

S križanjem ajd z različno višino stebel so dobili križance z običajno višino rasti in s stabilnim steblom. Križanci s številko 31/78 imajo dobro zgradbo rastlin, križanci s številkama 457/78 in 404/78 pa so ekološko dobro prilagodljivi in so lahko pomembni za uporabo pri nadaljnjem žlahtnjenju ajde. (Prevod uredništva).

Introduction

High-yielding and lodging-resistant varieties have been developed in some crops by the use of dwarfs. In our country, for example, for selection of winter wheat, forms of domestic dwarfs are used: dwarfs I and II, KMB-I (6, 4), in addition to Norin-10, the most widely used variety of winter wheat of foreign selection.

The varieties of rye, Malysh-72 (3) and Korotkostebelnaya-(short-stemmed)-69 (5) were obtained with the use of dwarfs.

There are practically no lodging resistant varieties of buckwheat, so when dwarf forms were found in 1972-1973 in Kamenets-Padolski Agricultural Institute (K-PAI) (dwarfs Nadezhda and Malysh) the question arose of how to use them in buckwheat breeding as donors for developing short-stemmed and lodging-resistant varieties. Later dwarfs were produced by A. N. Sabitov in the Bashkirian Research Institute of Agriculture and by N. V. Fesenko in the All-Union Research Institute of Cereal, Bean and Groat Crops (Orlovsky karlik) (2).

At present, there is a collection of buckwheat dwarfs in the Problem Research Laboratory for Buckwheat, K-PAI.

The aim of our studies was to evaluate breeding material obtained by crossing dwarf forms with long stem and high-branching ones.

Material and methods

The studies were performed from 1975-1982, in the Problem Research Laboratory for Buckwheat, K-PAI. Hybrids were obtained by crossing dwarfs with Altiramosum and reversed.

The dwarf Malysh is very short-stemmed (length 15-25 cm), often being a plant of spherical appearance ("cabbage").

A characteristic feature of the dwarf "Nadehzda" is a medium long stem (40-60 cm), 2-3 primary branches (no secondary branches).

Altiramosum is a recessive form characterized by high stem branching (primary branches begin from the 5-6th, sometimes 8th node) there are usually 4-5 branches. The stem is strong and elastic, its length reaches 140-200 cm (4).

To produce hybrids, the dwarf forms were sown together with a high-branching one, and with Altiramosum as a maternal form, plants with long-style flowers were sorted and artificial pollination was performed.

The F1 and F2 were studied in the hybrid nursery. After the hybrid plants had been analysed, plants of an intermediate type were selected. The material was studied in selection and control nurseries on $2m^2$ and $5m^2$ plots. The variety, Victoria, was used as a standard.

Phenological observations were made over the whole vegetation period. The yield was calculated in

g/m^2 in the selection nursery and in dt/ha in the control one. The grain quality was estimated by mass of 1000 seeds, filminesss and uniformity.

Hybridization of these forms was expected to produce plants with high stem branching and, from the dwarf form, a short stem and internodes and verticillate inflorescences (2, 4).

Results and discussion.

It was shown by the analysis of F2 and F3 that hybrids A x D (A-Altiramosum, D-dwarf) were most productive. Elite plants from this material were selected, characterized by a changed habitus or increased productivity (4).

After a double selection, promising plants in terms of yield productivity and grain quality were selected from the hybrid material; their evaluation was carried out in years with contrasting weather conditions. In the summer of 1979, there was a severe drought, some of the plants were lost, others produced little grain and were left for a repeat evaluation. Yield productivity was high, amounting to 26 g/m^2.

Some of the plants were further segregated into dwarf and Altiramosum. All deviating forms were discarded. In 1979, numbers 82/78 and 31/78 were of an intermediate type of plant (4) and increased yield productivity. Seven cultivars were of special interest for further selection (Table 1).

In 1980, with more moderate weather conditions, plants showed good growth and development. There were no segregations in the experimental cultivars under study. The field germination rate of seeds was on average 90-95%, survival rate 95-97%. The yield was 224-567 g/m^2.

On average over the 2 years, the best productivity characteristics were shown by experimental cultivars 82/78, 404/78, and 31/78; the last named being the most compact and lodging - resistant.

For the two years of study of the prospective experimental cultivars in the control nursery, their vegetation period averaged 78-81 days, in the variety Victoria - 80 days.

Promising cultivars were also highly productive in the control nursery, showing the highest productivity in 1982 (Table 2).

Experimental cultivar 31/78 gained on average 2.2 dt/ha, 457/78 - 2.6 dt/ha; cultivar 82/78 was especially productive in 1982, exceeding the standard by 5.8 dt/ha.

Thus, for two years, two cultivars, 457/78 and 82/78, were selected.

The technological grain properties of the cultivars under study were almost standard. In some years, their quality either exceeded the standard; in others it was higher than that in Victoria; the grain of cultivars 1062/76 and 496/76 had the thinnest hulls (Table 3).

Cultivar 31/78, which showed good architectonics, was given to the formation nursery.

An ecological test of the promising material was made in 1982 in a control nursery in the Oryol region (Table 4).

As seen from the data in Table 4, our cultivars were also promising in the Oryol region. The highest grain gain and high grain quality was noted in cultivar 404/78. This material has been used in further selection.

Table 1: Yield and quality of experimental cultivars in breeding nursery.

Indexes	Years	St.	404/78	117/78	1062/76	457/78	82/78	31/78	496/76
Productivity (g/m^2)	1979	26.0	37.0	30.0	34.0	11.0	45.0	43.0	31.0
	1980	198.0	401.0	224.0	249.0	331.0	567.0	558.0	343.0
	Mean	112.0	219	127.0	142.0	171.0	306.0	301.0	187.0
Mass of 1000 seeds (g)	1979	25.8	26.0	27.0	26.1	23.0	26.0	24.0	25.0
	1980	23.7	22.7	23.2	22.1	26.4	23.0	24.1	22.3
	Mean	24.8	24.4	25.1	24.1	24.7	24.5	24.1	23.7
Filminess (%)	1979	21.7	22.2	22.0	21.0	21.2	22.1	22.0	22.1
	1980	21.9	22.2	22.4	22.4	20.8	20.8	23.0	21.6
	Mean	21.8	22.2	21.7	21.7	21.0	21.5	22.5	21.8

Table 2: Relative productivity in control nursery.

Cultivar	Productivity (dt/ha)			Increased (dt/ha)		
	1981	1982	mean	1981	1982	mean
Victoria - St	9.5	13.3	11.4			
404/78	11.2	15.0	13.1	1.7	1.7	1.7
117/78	10.6	16.7	13.7	1.1	3.4	2.3
1062/78	11.2	15.2	13.2	1.7	1.9	1.8
31/78	11.8	15.3	13.6	2.4	2.0	2.2
496/76	11.1	15.7	13.4	1.6	2.4	2.0
457/78	11.9	16.0	14.0	2.4	2.7	2.6
82/79	12.1	19.1	15.6	2.6	5.8	4.2
			$LSD_{0.05}$	0.7	0.86	

Table 3: Yield and quality of cultivars in ecological test, 1982.

Cultivars	Mass of 1000 seeds (g)	Difference (+/-)	Filminess (%)	Difference (+/-)	Evennes (%)	Difference (+/-)
Victoria - St	26.9		22.1		76	
404/78	27.3	+0.4	22.6	-0.1	86	+10
117/78	26.5	-0.4	22.0	-0.1	78	+2
1062/76	26.4	-0.5	21.2	-0.9	75	-1
457/78	27.7	+0.8	21.1	-1.0	76	0
82/78	29.4	+2.5	22.0	-0.1	82	+6
31/78	27.7	+0.8	21.7	-0.4	86	+10
496/76	27.5	+0.6	21.3	-0.8	83	+7

Table 4: Yield and quality of cultivars in ecological test, 1982.

Cultivars	Yield of seed (dt/ha)	Increas (dt/ha)	Mass of 1000 seeds (g)	Filminess (%)	Seed size (%)	Hull percentage (%)
Shatilovskaya-5(st)	18.4		26.8	21.3	93.6	71.2
404/78	28.0	9.6	26.3	21.8	94.5	72.7
31/78	23.8	5.4	25.3	21.5	89.3	72.3
457/78	24.1	5.7	25.8	21.4	91.2	72.5

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Buckwheat as a dietary source of zinc, copper and manganese

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Key words: chromatography, correlation, gel filtration, micronutrients, trace elements

Abstract

The contents of zinc, copper and manganese in various samples of buckwheat were analysed. There was a variation in the contents of the trace elements among various buckwheat samples examined. In addition, a statistically significant positive correlation between these trace elements in buckwheat samples was observed. Chromatographic analysis indicated that there were some soluble components of zinc and copper in buckwheat flour, and that soluble manganese emerged as a single peak with the majority of soluble zinc. The present study suggests that buckwheat flour can contribute a considerable part of dietary zinc, copper and manganese.

Ajda kot prehranski vir cinka, bakra in mangana

Avtorji so analizirali vsebnost cinka, bakra in mangana v različnih vzorcih ajde. Ugotovili so raznolikost vsebnosti mikroelementov v vzorcih ter značilno pozitivno korelacijo med vsebnostmi različnih elementov. S kromatografsko analizo so ugotovili topne sestavine s cinkom in bakrom. Topen mangan se je pojavil kot en vrh s pretežnim delom topnega cinka. Na osnovi raziskave lahko sklepamo, da je ajdova moka možen vir cinka, bakra in mangana za prehrano ljudi. (Prevod uredništva).

Introduction

Zinc, copper and manganese are essential trace elements for humans. Zinc has many diverse physiological functions: it is required for the activity of many enzymes, and is important for the stability of biological membranes and for synthesis of nucleic acids, proteins and lipids (Lönnerdal et al. 1984). Copper is also important in many ways, e.g., it is required for the activity of several enzymes, and for hematopoiesis, cellular respiration, bone formation, and the cardiac function (McDowell 1992). Manganese is an activator of many enzymes and is essential for growth, reproduction, and skeletal development (Leach 1976). Nutritional deficiency of zinc and copper in humans is fairly common throughout the world (Prasad 1982, Williams 1982). Some researchers have also reported a deficiency of manganese in humans (Dahlstrom 1986, Doisey 1974). This area of nutrition research is thus currently receiving increased attention with the recognition of the importance of these trace elements. A recommended dietary allowance of these trace elements has been established in some countries in recent years. Various foods serve as dietary sources of these trace elements, but the nutritional properties of these trace elements in foods are still not well characterised.

Buckwheat (*Fagopyrum esculentum* Moench) is an important food in some areas of the world. Besides providing protein and energy, buckwheat may be a valuable source of minerals for the people who consume it. We have suggested that buckwheat can be potentially a good source of dietary zinc (Ikeda et al. 1990, Ikeda and Yamaguchi 1993). In addition, it appears that buckwheat may be a good source of dietary copper and manganese (Thacker et al. 1983, Amarowicz and Fornal 1987). However, these trace elements in buckwheat are still not fully characterised. For example, the contents of such trace elements in buckwheat may be generally dependent upon various factors such as a genetic factor, growth conditions and processing, but the detailed information involved is unavailable. On the other hand, there are a wide diversity of buckwheats, including various varieties and local varieties, available for human consumption. Thus characterisation of essential trace elements in buckwheat through systematic analysis of various samples of buckwheat is needed to clarify the role of buckwheat as a dietary source.

This study was undertaken to analyse the contents of zinc, copper and manganese in various samples of

buckwheat and to clarify the contribution of buckwheat as a dietary source of these trace elements.

Materials and methods

Materials: Twenty-six samples of buckwheat were selected for this investigation: eleven different kinds of common buckwheat seed (*F. esculentum*); eleven different kinds of commercial common buckwheat flour; and four flour fractions.

Three different kinds of buckwheat seed, i.e., Shinano No.1, Kiso-Zairai and Shinano-Natsusoba, which were cultivated at the Nagano Chushin Agricultural Experiment Station, Japan, were kindly provided by Dr. Hisayoshi Hayashi of the Station. Another kind of buckwheat seed, Kitawase, grown in Hokkaido, was kindly provided by Japan Agricultural Cooperatives, Hokkaido Shikaoi Branch. Four different varieties of buckwheat, i.e., Petra var., Darina var., Darja var. and Siva var., grown in Slovenia, were kindly provided by Dr. Ivan Kreft, Professor at Ljubljana University, Slovenia. Three different kinds of buckwheat seed, i.e., SXQ1, SXQ2 and SXQ3, grown in China, were kindly provided by Professor Lin Rufa at Shanxi Academy of Agricultural Sciences, China. These buckwheat seeds were milled in an electrically-driven mill before analysis.

Eight different kinds of commercial buckwheat flour were purchased from local markets at various places in Japan: buckwheat flour A was purchased in Iwate prefecture; flour B, in Ehime pref.; flour C, in Gifu pref.; flour D, in Kouchi pref.; flours E and F, in Tokushima pref.; flour G, in Nagano pref.; and flour H, in Hyogo pref. Three different kinds of commercial buckwheat flour sold in Slovenia were kindly provided by Dr. Ivan Kreft, Professor at Ljubljana University, Slovenia.

Four buckwheat flour fractions from a commercial mill were also used in this study: four flour fractions are successively obtained on milling the seed (Nagatomo 1984): the first fraction is superior flour; second, first flour; third, second flour; and the last, third flour.

Determination of trace elements: The contents of zinc, copper and manganese of samples were determined with a Hitachi 180-70 polarised Zeeman atomic absorption spectrophotometer. In determining trace elements in solid samples, the samples were wet-ashed with sulphuric acid and 30% hydrogen peroxide prior to atomic absorption spectrophotometry. In previous papers (Ikeda and Yamaguchi 1993, Ikeda and Shimizu 1993), we have reported the zinc content of various buckwheat samples, including some samples examined in the present paper; and in previous papers, the zinc content was expressed as mg on a fresh weight basis in relation to cooking and processing. In the present study, we have analysed zinc, copper and manganese by the above, newer type of atomic absorption spectrophotometer; and in the present paper, we judged it to be reasonable in view of clarifying the correlation between the trace elements that the contents of the trace elements were expressed as mg on a dry weight basis.

Solubility of trace elements and chromatography: To obtain information on the distribution of zinc, copper and manganese in buckwheat flour, the trace elements of the flour were classified with respect to solubility with 0.1 M Tris-HCl buffer (pH 8.0): buckwheat flour was extracted with 20-fold volume (V/W) of the above buffer for 2 h at 37°C, followed by centrifugation at 10,000 rpm for 20 min; the supernatant obtained was then assayed for trace elements. Another aliquot of the supernatant was applied on a Sephadex G-50 column (1.6 x 95 cm), which had been pre-equilibrated against 0.1 M Tris-HCl buffer (pH 8.0).

Other analyses: Moisture content was determined by a YMC Cho-Balance IB-30 infrared moisture analyser. The distribution of protein in column effluents was determined by means of A_{280} measurements. Data were subjected to statistical analysis of variance and the significance of difference was determined by the t-test.

Results and discussion

Contents of zinc, copper and manganese in buckwheat

Table 1 shows the contents of zinc, copper and manganese in various buckwheat seeds. There was a variation in zinc content among various buckwheat seeds examined, with a range from 1.37 to 2.73 mg per 100 g flour. There was also a variation in copper content among buckwheat seeds examined (Table 1), with a range from 0.41 to 0.68 mg per 100 g flour. On the other hand, a larger variation in manganese content with high coefficient of variation among buckwheat seeds examined was observed (Table 1). In addition, the content of manganese in buckwheat seeds grown in Slovenia was significantly ($p<0.05$) higher than those grown in both Japan and China.

Table 2 shows the contents of zinc, copper and manganese in various commercial buckwheat flours. There was a variation in the contents of three trace

Table 1: Contents of zinc, copper and manganese in flour for various buckwheat seeds.

Buckwheat samples	Zinc	Copper	Manganese
	mg/100g flour (dry weight basis)		
Buckwheat seeds grown in Japan			
Shinano No. 1	2.09 ± 0.02^{b}	$0.52 \pm 0.05^{c\,d}$	0.84 ± 0.01^{g}
Kiso-Zairai	$1.87 \pm 0.03^{f\,g}$	0.41 ± 0.02^{g}	1.02 ± 0.02^{e}
Shinano-Natsusoba	$1.90 \pm 0.02^{e\,f}$	$0.42 \pm 0.04^{f\,g}$	0.91 ± 0.01^{f}
Kitawase	1.37 ± 0.02^{i}	$0.51 \pm 0.03^{d\,e\,f}$	0.59 ± 0.02^{j}
Buckwheat seeds grown in Slovenia			
Petra	2.73 ± 0.04^{a}	0.60 ± 0.01^{b}	1.60 ± 0.01^{b}
Darina	1.83 ± 0.03^{g}	$0.60 \pm 0.05^{b\,c}$	1.46 ± 0.03^{c}
Darja	1.52 ± 0.02^{h}	$0.49 \pm 0.02^{d\,e}$	1.21 ± 0.01^{d}
Siva	1.96 ± 0.01^{d}	0.68 ± 0.04^{a}	1.79 ± 0.02^{a}
Buckwheat seeds grown in China			
SXQ1	$1.98 \pm 0.04^{c\,d}$	0.57 ± 0.02^{c}	0.73 ± 0.02^{i}
SXQ2	2.02 ± 0.03^{c}	0.61 ± 0.02^{b}	0.82 ± 0.01^{h}
SXQ3	$1.93 \pm 0.05^{d\,e}$	0.52 ± 0.01^{d}	0.81 ± 0.02^{h}
Means (n = 11)	1.93 ± 0.34	0.54 ± 0.08	1.07 ± 0.39
CV (%)	17.6	14.8	36.4

Values are means ± S. D. (n = 4).
Values within a column that do not share a common superscript are significantly different at $p < 0.05$.
CV: Coefficient of variation.

Table 2: Contents of zinc, copper and manganese in various commercial buckwheat flours.

Buckwheat flour	Zinc	Copper	Manganese
	mg/100g flour (dry weight basis)		
Buckwheat flour available in Japan			
Flour A	1.79 ± 0.02	0.42 ± 0.02	1.78 ± 0.01
B	1.33 ± 0.01	0.36 ± 0.03	1.04 ± 0.02
C	0.70 ± 0.01	0.35 ± 0.05	0.46 ± 0.02
D	1.70 ± 0.05	0.45 ± 0.02	1.26 ± 0.03
E	2.17 ± 0.01	0.52 ± 0.01	1.63 ± 0.03
F	2.79 ± 0.03	0.62 ± 0.01	2.98 ± 0.02
G	1.61 ± 0.03	0.52 ± 0.01	1.09 ± 0.01
H	2.33 ± 0.02	0.56 ± 0.02	1.13 ± 0.02
Means (Japanese flours, n = 8)			
	1.80 ± 0.64	0.48 ± 0.10	1.42 ± 0.75
Buckwheat flour available in Slovenia			
Flour A	3.95 ± 0.02	0.64 ± 0.01	3.34 ± 0.06
B	2.09 ± 0.07	0.53 ± 0.02	0.80 ± 0.01
C	4.47 ± 0.06	0.62 ± 0.02	2.94 ± 0.05
Means (Slovenia flours, n = 3)			
	3.50 ± 1.25	0.60 ± 0.06	2.36 ± 1.37
Means (whole, n = 11)	2.27 ± 1.11	0.51 ± 0.10	1.68 ± 0.98
CV (%)	48.9	19.6	58.3

Values are means ± S.D. (n = 4).
CV: Coefficient of variation.

Table 3: Contents of zinc, copper and manganese in four buckwheat flour fractions.

Buckwheat flour fraction	Zinc	Copper	Manganese
	mg/100g flour (dry weight basis)		
Superior flour	0.43 ± 0.01	0.32 ± 0.02	0.38 ± 0.01
First flour	0.29 ± 0.01	0.37 ± 0.02	0.29 ± 0.01
Second flour	0.83 ± 0.02	0.46 ± 0.04	0.66 ± 0.02
Third flour	3.64 ± 0.03	0.78 ± 0.03	2.74 ± 0.01

Values are means ± S.D. (n = 4).

Table 4: Correlation coefficients among zinc, copper and manganese in various buckwheat samples.

Buckwheat Samples	Correlation coefficient between two minerals		
	Zn to Cu	Zn to Mn	Cu to Mn
All buckwheat samples in Tables 1 to 3 (n = 26)	0.770**	0.862**	0.675**
All buckwheat seeds and commercial buckwheat flours in Table 1 and 2 (n = 22)	0.645**	0.820**	0.539**
Commercial buckwheat flours in Table 2 (n = 11)	0.865**	0.873**	0.756**
Buckwheat flour fractions in Table 3 (n = 4)	0.983*	0.999**	0.983*

* Significant correlation at $p < 0.05$.

** Significant correlation at $p < 0.01$.

elements: a high variation with high coefficient of variation was found with manganese and zinc; and a low variation with low coefficient of variation, with copper. On the other hand, the buckwheat flours of Slovenia, with the exception of some samples, contained a relatively higher level of zinc as compared with those of Japan. In addition, buckwheat flours of Slovenia, but with the exception of some samples, contained a relatively higher level of manganese as compared with those in Japan. No marked difference in copper content between Japan and Slovenia was observed (Table 2).

Table 3 shows the contents of zinc, copper and manganese in four buckwheat flour fractions. There were marked differences in the contents of three trace elements among four buckwheat flour fractions examined. The highest content of trace elements was found with the third flour.

Table 4 shows correlation coefficients among zinc, copper and manganese contents in various samples of buckwheat. Interestingly, there were significant ($p<0.05$ or 0.01) positive correlations (Table 4) between each two of the three trace element contents, i.e., zinc to copper, zinc to manganese, and copper to manganese, with buckwheat samples examined. These findings suggest that the three trace elements may have a similar distribution within the buckwheat kernel, but the detailed information remains unavailable. In wheat, some researchers (Lorenz et al. 1980, Dikeman et al. 1982, Iskander et al. 1987) have reported a correlation between the contents of some minerals in various wheat flours.

Although the exact reason for the observed variations in the contents of zinc, copper and manganese among buckwheat samples (Tables 1 to 3) is still obscure, the contents of nutrients, including trace elements, in buckwheat, may be generally influenced by many factors, such as genetic factors, environmental conditions, and processing conditions. In this connection, the following three findings in the present study are interesting: firstly, there was a variation in the contents of trace elements, especially of manganese, among buckwheat samples (Tables 1 and 2); secondly, buckwheat samples of Slovenia had a relatively higher level of manganese and zinc than those of Japan (Tables 1 and 2); and lastly, the three trace elements were positively correlated among each other (Table 3). In other cereals such as wheat and rice, various factors responsible for variations in mineral content are the subject of controversy (El-Gindy et al. 1957, Zook et al. 1970, Taira 1975, Peterson et al. 1983). Further research is needed to

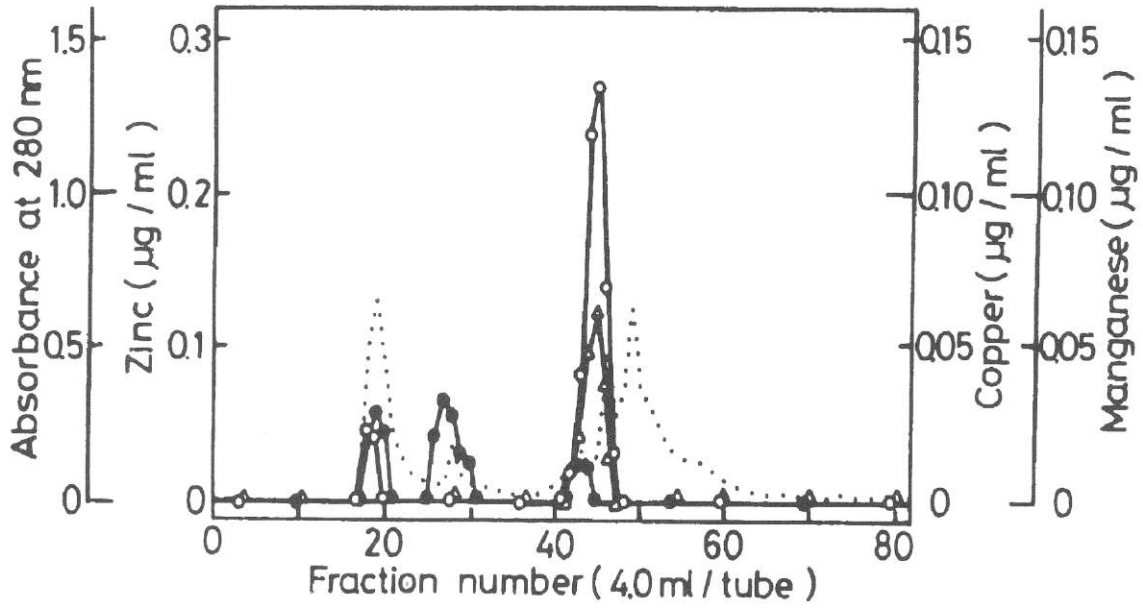

Figure 1: Chromatographic elution profile of a buffer-extract of buckwheat flour on Sephadex G-50: ○ zinc, ● copper, △ manganese, and ..... absorbance at 280 nm.

analyse factors responsible for the mineral content of buckwheat in relation to mineral nutrition.

Chemical forms of zinc, copper and manganese in buckwheat

Figure 1 shows the chromatographic elution profile of a buffer-extract of buckwheat flour on Sephadex G-50. The soluble zinc comprised 50.8±1.8% of its total amount; copper, 67.6±5.1%; and manganese, 35.3±2.4%. The soluble zinc consisted of two peak fractions (Figure 1): one main peak fraction had a molecular weight of approximately 1 to 2 kDa; and the other peak fraction, a molecular weight of 30 kDa or over. This elution profile of zinc was essentially similar to that reported in previous papers (Ikeda et al. 1990, Ikeda and Shimizu 1993). On the other hand, the soluble copper consisted of three peak fractions (Figure 1): each peak fraction had a molecular weight of approximately 2 kDa, 15 kDa and 30 kDa or over, respectively. Furthermore, the soluble manganese emerged as a single peak with a molecular weight of approximately 1 to 2 kDa. The manganese was eluted at the same position of the zinc main peak fraction. These findings suggest that a zinc-bound component of buckwheat may be identical with a manganese-bound component of buckwheat.

Buckwheat as a dietary source of zinc, copper and manganese

Buckwheat contains a relatively higher level of zinc, copper and manganese (Tables 1 and 2) as compared with other cereal foods such as polished rice, wheat flour and corn (O'Dell et al. 1972, Resources Council 1991). Thus we attempted to estimate the contribution of buckwheat dishes as a dietary source of zinc, copper and manganese based on the data of the present findings (Tables 1 and 2). Recommended dietary allowances (RDA) for zinc have been established in several countries: the RDA of zinc for adults range from 7 to 15 mg per day (RDAJ 1991). The RDA for copper and manganese have been recently established in some countries such as USA (RDAJ 1991): the RDA of copper for adults range from 1.2 to 3 mg per day; and the RDA of manganese, from 2 to 5 mg per day. On the other hand, there are many buckwheat dishes around the world. We have estimated the contribution to dietary trace elements of some buckwheat dishes without soaking: these dishes include "Soba-Gaki" in Japan and "Zlevanka" in Slovenia. On the assumption that one serving of such buckwheat dishes is usually made from approximately 80 g of buckwheat flour, the dish was estimated to provide about 1.5 mg of zinc, 0.38 mg of copper and 1 mg of manganese on a fresh weight basis for

consumption. Therefore our estimation indicates that one serving of a buckwheat dish may potentially provide approximately 10 to 20% or the RDA for zinc, 10 to 30% of RDA for copper, and 20 to 50% of RDA for manganese. In conclusion, buckwheat may be an important source of dietary zinc, copper and manganese.

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Technological and qualitative characteristics of food products made with buckwheat

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Key words: bread, extrusion products, pasta

Abstract

Various bread, pasta and extrusion samples were prepared with differing amounts of buckwheat flour blended with other cereals. Both the technological properties of the raw flours and the qualitative characteristics of the final products were studied. The nutritional aspect of the final products was also taken into account, and thus their approximate composition and aminoacid profiles were studied.

Tehnološke in kakovostne lastnosti prehranskih izdelkov iz ajde

Kruh, testenine in ekstrudirani vzorci so bili pripravljeni z različnimi količinami ajdove moke v kombinaciji z mokami drugih žit. Avtorja sta raziskovala tehnološke lastnosti mok in kakovost končnih izdelkov. S prehranskega vidika je bila raziskovana aminokislinska sestava končnih izdelkov ter vsebnost beljakovin in vlaknin.

Introduction

In regions where buckwheat is cultivated and used for human consumption, it is used today in simple traditional dishes.

However, in recent years, interest in buckwheat has been growing, especially due to the composition of its proteins, characterised by a high lysine content and absence of glutins, and particularly with a view to producing foods for a specific category of consumer, patients with coeliac disease.

Another point of interest is that, as the industrialised countries turn towards "biological agriculture", the use of buckwheat products is being re-evaluated, since it is a hardy plant and no synthetic chemicals are used in its cultivation. The aim of the present study is to examine the technological properties of buckwheat flour, with the standard instruments used for wheat, in order to obtain additional information to that published (1-7), how buckwheat can be used in food processing for human consumption, as a single ingredient or blended with other cereals.

Three different products were considered: bread, pasta and extrusions. Buckwheat flour was used both blended with other cereals and as a single ingredient. Chemical analyses and rheological tests were carried out on the raw flours and on the final products; the aminoacid composition of the extrusions and pasta made with buckwheat, blended with other cereals or as the sole ingredient, was also studied.

Materials and methods

In order to give a clear picture of the study, the analytical methodologies are given separately for each single type of product; in fact, specific technological analyses were carried out for each final product.

BREAD

Flour blends using increasing amounts of buckwheat flour (0%, 15%, 30% and 50%), a local variety from Bolzano, Italy, and a blend of Centauro and Mec wheat varieties, were used for the following tests:

Alveograph: An MA82 Alveograph model was used to determine the alveograph according to the original Chopin methodology; 250 g of flour were used and the W,G, and P/L values are given according the Chopin methodology. A pneumatic Buhler MLU202 mill was used to break down and grind each of the samples three times.

Farinograph: A Brabender farinograph was used, which measures certain physical properties of the

flour by measuring the energy required to obtain a dough.

Falling Number (ISO n. 3093): This was determined on 7 g of flour according to the Hagberg and Perten technique and a 1600 Falling Number instrument was used. Results are expressed in seconds. This parameter is used to determine the alpha-amylasic activity of the grains, which is particularly high in germinated or germinating caryopses. This method involves the rapid gelatization of a flour suspension steeped in boiling water and the measurement of the resulting liquefaction of the amino content of the sample.

Bread-Making Test: The standard method employed at the Istituto Nazionale della Nutrizione for bread-making tests was used.

Formula:	Flour	500.0 g
	Salt	7.5 g
	Yeast	20.0 g
	Water	200.0 ml

Processing Time:	I Dough	5 minutes
	I Rising	(T=30°C; 80% humidity) 30 minutes
	II Dough	3 minutes
	II Rising	(T=30°C; 80% humidity) 45 minutes
	Baking	30 minutes at 240°C

Volumetric Weight: In the Institute's laboratories, the volumetric weight of bread is determined by the amount of colza seeds required for the volume the bread takes up in a calibrated dish, bearing in mind that 1000 cm^3 of colza seeds is taken as weighing 660 grams.

Determination of how hard the soft part of the bread is: A Universal Testing Machine was used. With this method (AACC n. 74-90), a 25 mm thick slice of bread is compressed up to 25% using a piston, 36 mm in diameter, at a speed of 100 mm/min. The results, expressed in Newtons, indicate the amount of power, required for compression and how soft the bread is.

PASTA

Thin 1mm thick spaghetti were made, using a PAT press with a capacity of 50 kg/h, to run tests on.

Production process:

Water temperature	50°C
Head temperature	45°C
Cylinder temperature	25°C
Vacuum	720 mm Hg
Head pressure	60 Bar
Water percentage	36.00%

A large amount of water was used so that it would be easy to work the dough.

Drying process:

Type of Process	= HT static cell
Final moisture	10.13%

Cooking quality: Both a sensorial method to evaluate the stickiness and the consistency of the pasta when squeezed and its stiffness, as well as a chemical method to determine the starchy organic substance which excludes from the sample during cooking were used.

EXTRUSIONS

A Pavan-Mapimpianti TT58W bi-screw extruder with 58mm diameter screws was used to produce extrusions for the study.

Pre-cooked flours made from 100% buckwheat and rings made from both 100% buckwheat and buckwheat blended with other cereals were produced.

Cereal rings 1 (T of extrusion 160/190°C):	
buckwheat	40%
sugar	20%
wheat	25%
rice	15%
Cereal rings 2 (T of extrusion 140/160°C):	
buckwheat	40%
sugar	20%
wheat	25%
rice	15%
Buckwheat rings:	
buckwheat	79.6%
sugar	20%
malt	0.4%

The basic chemical composition and aminoacid composition of the samples were studied.

Table 1: Rheologic characteristics of flours blended with buckwheat for bread-making (W, G, P/L values are according to the Chopin methodology. A is capacity of water binding. B and CD are respective times of dough development and stability. U.F. are farinographic units.).

	Control flour 0% buckwheat	Control flour + 15% buckwheat	Control flour + 30% buckwheat	Control flour + 50% buckwheat
Alveograph				
Moisture %	13.20	13.20	13.20	13.30
W	181.00	114.00	84.00	93.00
G	22.10	17.82	13.07	9.87
P/L	0.55	0.79	1.69	4.92
Farinograph				
A (%)	53.50	53.50	53.40	55.20
B (min)	1'15"	1'	1'	5'30"
CD (min)	1'45"	2'15"	8'30"	5'30"
E_{10} (U.F.)	70.00	40.00	40.00	40.00
Falling Number (sec)	367.00	288.00	278.00	260.00
Volumetric Weight (cm^3)	653.00	720.00	693	544.00
Instrom (Newton)	14.2 (±2.5)	14.4 (±1.7)	15.1 (±1.9)	19.3 (±2.1)

Result and discussion

BREAD

The aim of the bread-making tests was, above all, to check how the addition of varying amounts of buckwheat affected the technological properties of wheat flour. To obtain various blends of flour, increasing amounts of buckwheat flour (15%, 30% and 50%) were added to a control flour, a blend of Centauro and Mec varieties, which have good bread-making properties. The results of the rheologic values obtained are given in Table 1 and the relative alveograph and farinograph plots are given in Figures 1 and 2.

Alveograph: The comparative flour (0% buckwheat) had good alveograph values and appeared well-balanced and suitable for bread-making. The W value, in terms of the strength of the flour and expression of the quality of the protein network, was 181 x 10-4 joule, while the P/L ratio, which indicates the relation between the tenacity and spreading capacity of the dough, was 0.55.

As the amount of buckwheat in the blends increased, the W value decreased and the P/L ratio became more and more unbalanced, up to a maximum of 4.92. Dough containing 50% buckwheat was found to be "lumpy" and very difficult to work during the bread-making process.

Farinograph: In contrast to the alveograph results, the addition of up to 30% of buckwheat appears to increase the resistance and stability of the dough.

Falling Number: Given that values around 250 sec. are considered optimum in bread-making, the addition of buckwheat improved the values of this parameter. The control flour, having a value higher than 300 sec., definitely needed the addition of malt or malted flours in order to increase the alpha-amylasic activity. A similar effect was obtained with the addition of 15-50% of buckwheat flour.

Table 2: Approximate chemical composition of buckwheat pasta.

	Type 1	Type 2	Type 3
Moisture %	10.30	10.00	9.20
Protein (% d.m.)	12.20	11.30	10.80
Ash (% d.m.)	2.41	2.18	1.66
Total dietary fibre (% d.m.)	5.19	4.98	3.42
Insoluble	4.13	3.96	2.30
Soluble	1.06	1.02	1.12
Percentage soluble fibre	20.40	20.50	32.7
Tannins	0.28	0.24	0.12
TOM:			
Stickiness	n.d.	n.d.	30.00
Firmness	n.d.	n.d.	30.00
Bulkiness	n.d.	n.d.	30.00
% Organic matter	n.d.	n.d.	2.40

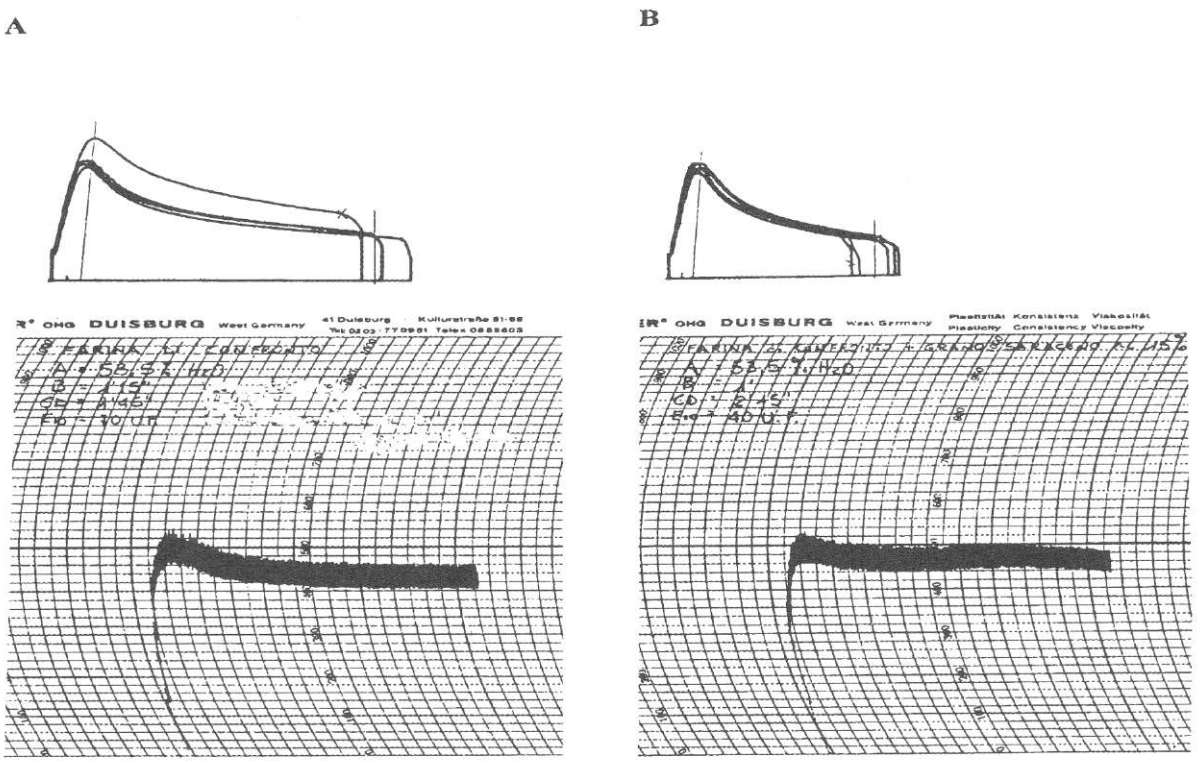

Figure 1: Alveograph and farinograph of flour blends with varying amounts of buckwheat: (A) control flour + 0% buckwheat and (B) control flour + 15% buckwheat.

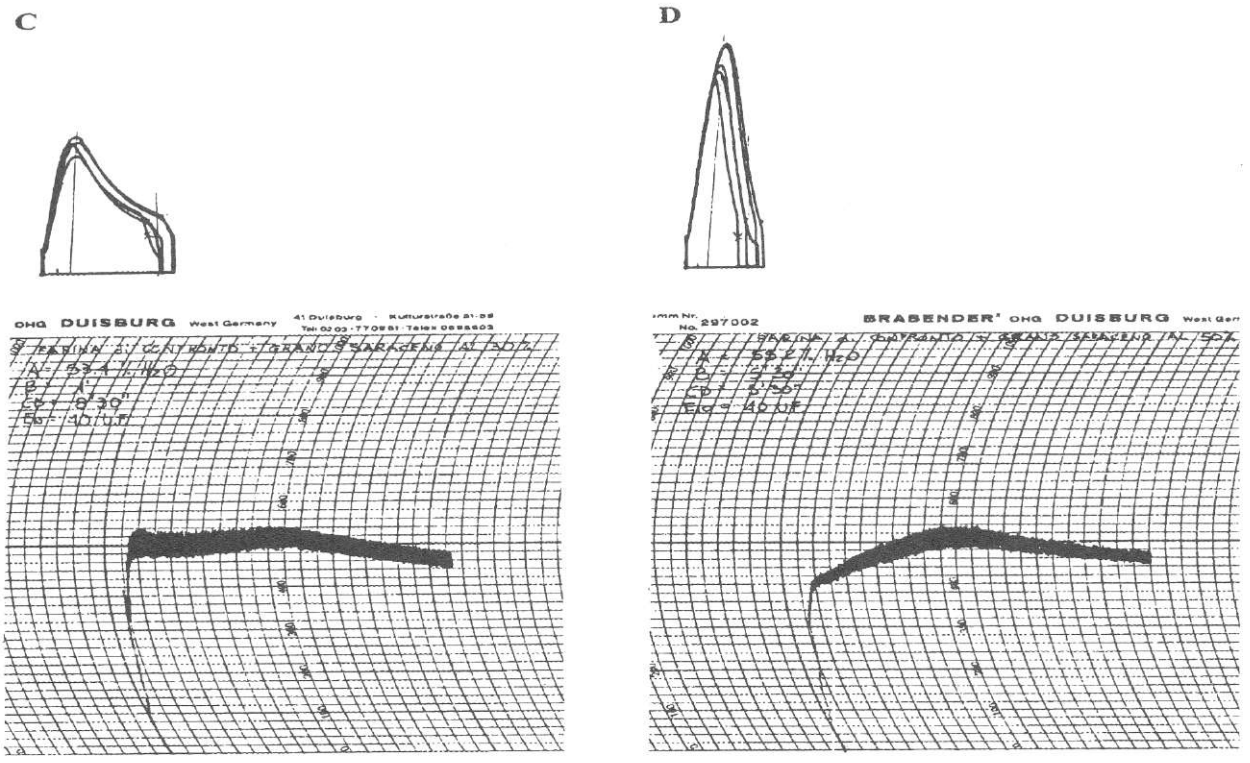

Figure 2: Alveograph and farinograph of flour blends with varying amounts of buckwheat: (C) control flour + 30% buckwheat and (D) control flour + 50% buckwheat.

Figure 3: Bread made with varying amounts of buckwheat flour: (1) 0% buckwheat, (2) 15% buckwheat, (3) 30% buckwheat and (4) 50% buckwheat.

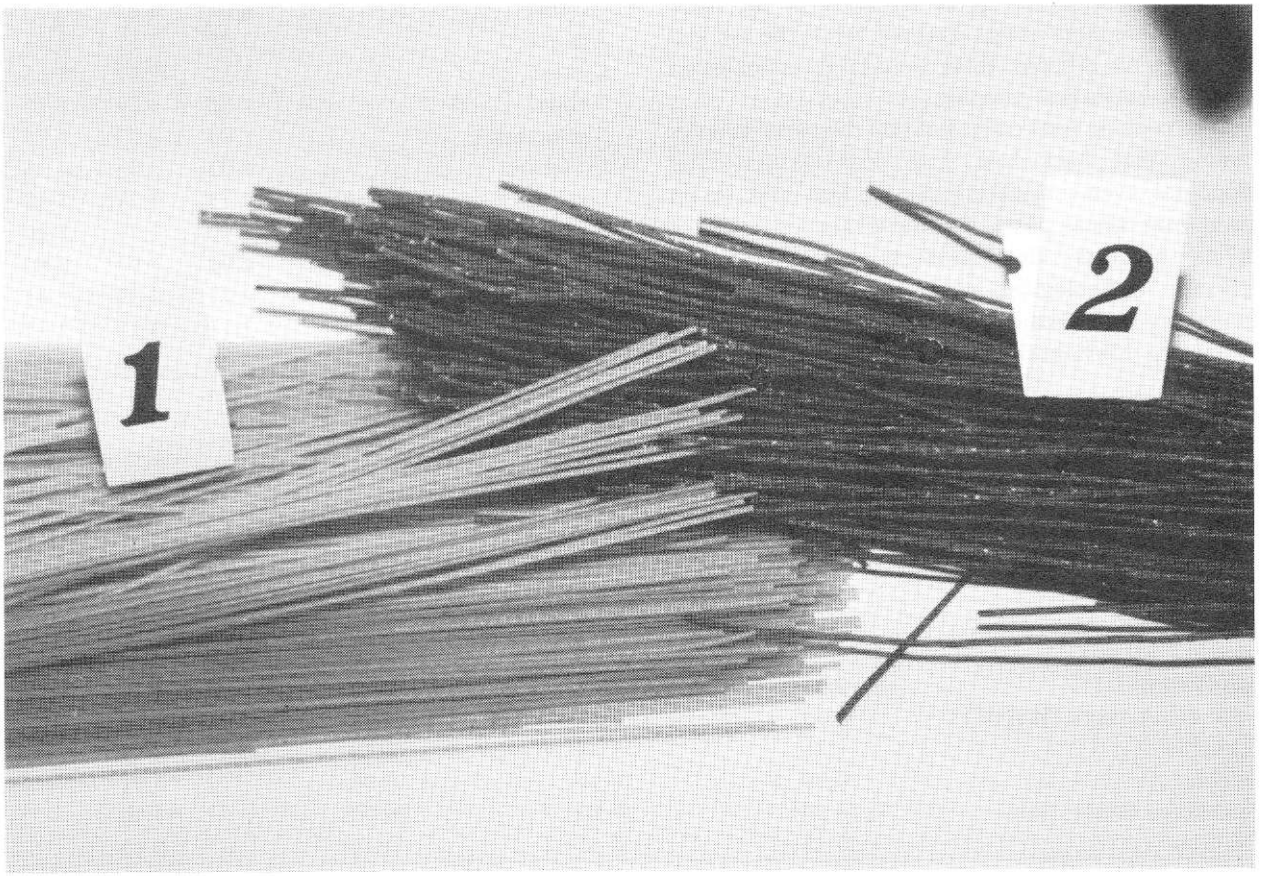

Figure 4: Pasta produced with varying amounts of buckwheat flour: (1) 50% wheat + 50% buckwheat, (2) 100% buckwheat (including 55% pre-gelatinized flour).

Bread-Making Tests: The results of bread-making tests are given in Figure 3. With up to 30% buckwheat, the bread rose well; the volumetric weight was not much different from the control flour as regards the absolute value. However, bread containing 50% buckwheat did not rise and lacked porosity. This is all confirmed by the values from an Instrom, which is used to measure how hard the soft part of the bread is: the values for bread made with 15% and 30% buckwheat were similar to those for bread made with control flour.

PASTA

Three types of pasta were made for testing:
1 Type: 100% buckwheat
2 Type: 100% buckwheat, of that 55% pre-gelatinized buckwheat flour
3 Type: 50% buckwheat + 50% wheat

The approximate values of the three samples under examination are given in Table 2.

The humidity and protein values in all three samples were more or less constant, while the ash and fiber contents differed: there were larger amounts in the 100% buckwheat samples.

The cooking tests carried out on the pasta samples showed that buckwheat had poor pasta-making properties, even considering that the tests were set up for durum wheat pasta, for the characterization of Italian products; thus products with completely different technological characteristics than those containing buckwheat. In the first two types of pasta, containing 100% buckwheat, a qualitative judgement was not even possible. The final product obtained from the sample containing 50% wheat and 50% buckwheat flour was very sticky, the venation poor, and it was also very dense; the determination of the organic substance present in the cooking water was very high.

EXTRUSIONS

Results of tests on the chemical composition of buckwheat extrusion products and their amino-acid composition are presented in Tables 4 and 5.

Table 3: Aminoacid composition (g/100g of protein) of buckwheat pasta.

	Type 1	Type 2	Type 3
Aspartic acid	5.58	7.88	5.03
Threonine	2.98	3.40	2.94
Serine	5.09	4.99	5.22
Glutamic acid	30.88	20.63	29.87
Proline	13.45	7.38	17.66
Glycine	4.08	4.83	4.17
Alanine	3.50	4.82	3.68
Cystine	2.53	2.62	2.65
Valine	4.85	4.69	5.11
Methionine	1.97	2.23	1.75
Isoleucine	4.07	3.58	4.35
Leucine	7.23	7.23	7.50
Tyrosine	2.90	2.77	3.20
Phenylalanine	4.91	4.91	5.80
Lysine	4.17	4.39	2.41
Histidine	2.11	3.13	2.37
Arginine	5.20	7.28	4.30

Table 4: Approximate chemical composition of buckwheat extrusion products.

	Pre-cooked flour	Cereal rings 1	Cereal rings 2	Cereal rings 3
Moisture (%)	5.60	2.80	2.40	2.60
Protein (% d.m.)	11.40	8.80	8.40	8.30
Ash (% d.m.)	2.36	2.41	2.11	2.12
Total dietary fibre (% d.m.)	5.15	4.12	4.78	4.72
Insoluble	4.36	3.18	3.79	3.81
Soluble	0.79	0.94	0.99	0.91
Percentage soluble fibre	15.30	22.80	20.70	19.30
Tannins	0.25	0.16	0.10	0.12

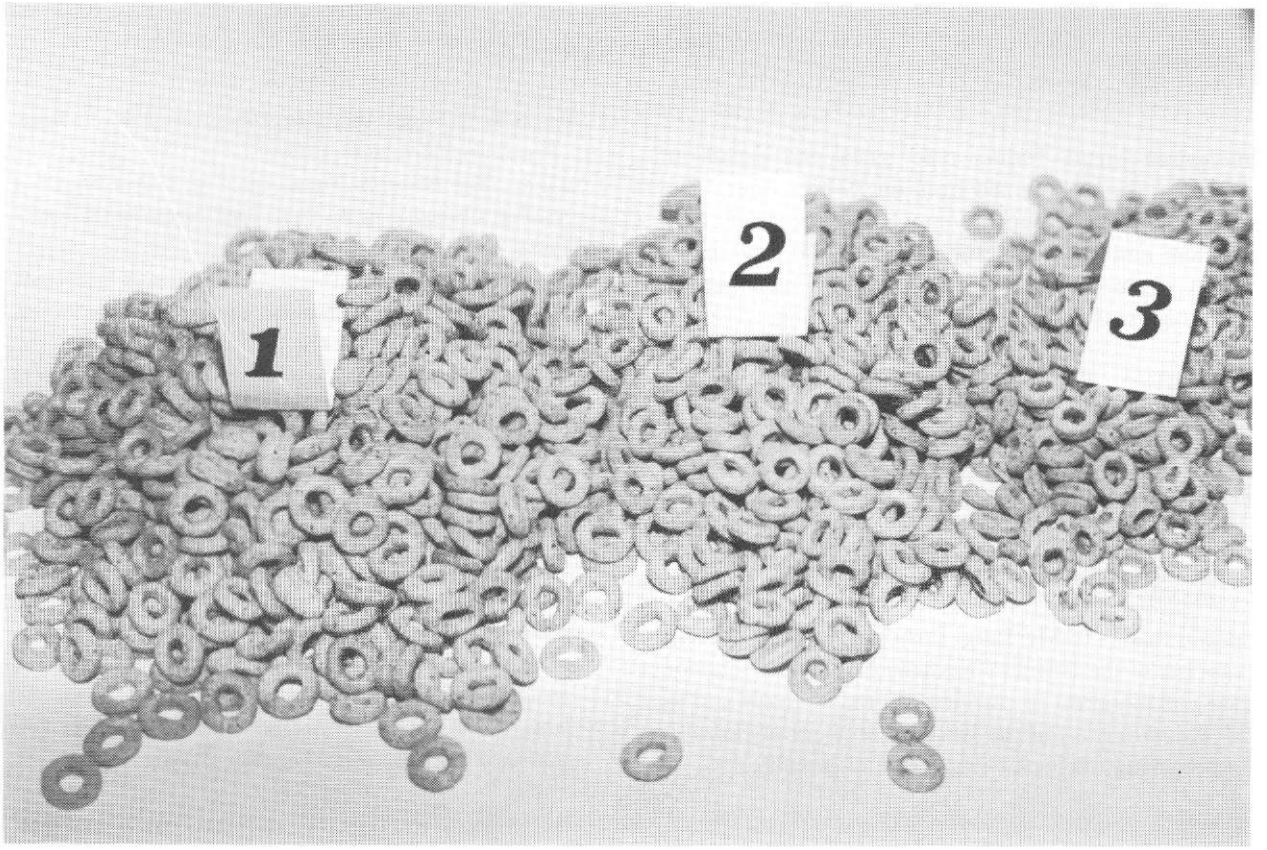

Figure 5: Extrusions made with 100% buckwheat blended with other cereals:

(1) Cereal rings (T of extrusion 160/190°C)		(2) Cereal rings (T of extrusion 140/160°C)		(3) Buckwheat rings	
buckwheat	40 %	buckwheat	40 %	buckwheat	79.6 %
sugar	20 %	sugar	20 %	sugar	20.0 %
wheat	25 %	wheat	25 %	malt	0.4 %
rice	15 %	rice	15 %		

Table 5: Aminoacid composition (g/100g of protein) of buckwheat extrusion products.

	Pre-cooked flour	Cereal rings 1	Cereal rings 2	Cereal rings 3
Aspartic acid	8.94	8.46	7.53	7.52
Threonine	3.57	3.49	3.34	3.26
Serine	4.71	4.81	4.96	4.91
Glutamic acid	20.39	21.41	25.03	24.46
Proline	6.30	6.40	8.25	8.05
Glycine	5.18	5.05	4.69	4.61
Alanine	4.50	4.39	4.14	4.10
Cystine	2.47	2.48	1.86	2.58
Valine	5.80	5.73	5.71	5.84
Methionine	2.15	2.13	1.63	1.63
Isoleucine	4.46	4.15	4.43	4.62
Leucine	8.15	7.80	8.75	7.91
Tyrosine	2.72	2.99	4.21	4.14
Phenylalanine	4.81	5.18	5.03	5.43
Lysine	5.25	4.52	4.39	4.12
Histidine	3.05	2.64	3.53	3.11
Arginine	7.98	7.75	10.33	6.65

Conclusion

Interesting bread, Italian type pasta and extrusion products may be obtained from buckwheat flour. The good chemical composition of buckwheat and excellent amino-acid profile of buckwheat proteins is mainly retained in products, despite some blending of buckwheat with other cereals and processing.

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Proximate chemical composition and protein characterization of the buckwheat cultivated in Italy

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Abstract

In this study, samples of buckwheat from various regions of Italy were analysed. In addition to the basic chemical composition, the protein fractions were studied for a possible characterization of the varieties cultivated in Italy. The analyses carried out did not present characteristic protein electrophoretic bands, but showed that there were greater differences within domestic varieties than among them. The approximate chemical composition of the buckwheat studied indicates that varieties cultivated in Italy do not differ much from varieties cultivated in other countries of Europe.

Kakovost beljakovin in kemijska sestava ajde iz Italije

Raziskovani so bili vzorci ajde iz različnih predelov Italije. Poleg osnovne kemijske sestave so bile raziskovane tudi beljakovinske frakcije, da bi se ugotovilo eventualne značilnosti vzorcev ajde pridelanih v Italiji. Elektroforetska analiza beljakovin zrn je pokazala večje razlike znotraj vzorcev kot med njimi. Rezultati kemijskih analiz vzorcev zrnja ajde iz Italije so podobni, kot so znani za vzorce ajde iz drugih evropskih dežel.

Introduction

Buckwheat (*Fagopyrum esculentum* Moench), a dicotyledonous plant, which belongs to the *Polygonaceae* family, is usually considered a cereal in agriculture and food technology because of its usage and the cultivation techniques used. Originating from Asia and introduced into Europe around the 15th century, the cultivation of buckwheat has spread to Canada, the United States of America and to certain areas of Africa and Latin America, with an annual yield, worldwide, of approximately one million tons. The agricultural features which have first and foremost encouraged its cultivation in such varied regions are primarily that buckwheat is semi-wild, does not have particular soil or fertilization requirements and can, furthermore, grow at high altitudes (above 3,000 metres in Nepal and Bhutan).

In Italy, buckwheat is cultivated in various areas in the Alps as a second annual crop, usually following rye. It is sown in July and harvested at the end of September. The flour obtained from husked buckwheat is used in typical regional dishes such as polenta, canederli and buckwheat pasta - pizzocheri.

The buckwheat varieties grown in Italy do not have a constant and characteristic genotype. Buckwheat is present in various different varieties produced locally and reproduced annually. In comparison with traditional cereals, buckwheat proteins are high in lysine, which makes it interesting from a nutritional point of view though a protein digestibility reduction due to the high fiber and tannins content can be observed (Pomeranz and Robbin 1972, Pomeranz et al. 1975, Eggum 1980, Javornik et al. 1981). An interesting feature is represented by the lack of gluten, due to the almost total absence of prolamin. It could therefore be used as a substitute for wheat in gluten-free diets for coeliac patients.

The aim of this article is to characterize the protein fractions of certain varieties of buckwheat cultivated in Italy in order to evaluate the quality and to see whether it would be possible to characterize buckwheat by production area.

Materials and methods

Samples

Five samples of buckwheat which typify the various areas of production were analyzed. The selected samples came from the following geographical areas of Italy:

Brixen (Bolzano),
Klause area (Bolzano),
Villanders (Bolzano),
St. Leonard (Bolzano),
Teglio (Sondrio).

The samples were husked manually and the whole kernel, deprived of its external hull, was removed and used for all the analyses. A portion of the sample was milled using a Cyclotec laboratory Mill with a 1 mm mesh sieve.

Proximate Chemical Composition Analyses

Proximate chemical composition of the samples under investigation was performed following the Official Methods of Analysis used for wheat. Moisture was determined on a sample dessicated in an oven at 105°C up to constant weight. The residual ash was obtained by incinerating the sample in a muffola at 575°C. The Kjeldahl method was applied to determine protein content. The nitrogen value obtained was multiplied by 5.75. Fats were determined by solvent extraction in a Soxhlet extractor.

Total dietary fiber was determined according to the AOAC method (AOAC 1990). Samples were digested by enzymes (heat stable amylase, protease and amyloglucosidase) to remove proteins and starch. Fiber was then precipitated with ethanol, filtered and dried. The residue weighed and subtracted from the residual ash and proteins represented the total fiber content. The insoluble and soluble fractions were determined by applying a modification of the AOAC method (Prosky et al. 1988).

The value of "the 1000 seed weight" to determine yield and maturation rate of buckwheat was taken into consideration. Tannin content was determined using the vanillin - HCl method (Burns 1971). All analyses were repeated three times.

Protein separation and aminoacid identification

Firstly, proteins were separated according to their solubility characteristics using the method proposed by Osborne. Samples were hydrolysed with 6 N HCl for 24 hours and 72 hours to determine the aminoacid composition. A separate hydrolysis was carried out for methionine and cystine. An ion-exchange system was used for chromatographic analysis (Spackman et al. 1958).

For electrophoresis, total seed proteins were extracted with 0.14 M Tris-HCl electrophoresis buffer (pH 6.8), 4% SDS, 3% 2-mercaptoethanol. An SDS-PAGE vertical electrophoresis was carried out on the total proteins, using the modified Leammli method (1970), the separating gel had a final concentration of 10% acrylamide, and the stacking gel contained 3% acrylamide. Tris-glycine buffer (0.025 M Tris and 0.192 M glycine) and both parts of the gel contained 0.1% SDS.

Results and discussion

Proximate chemical composition of the buckwheat samples is given in Table 1. The weight of 1,000 seeds of buckwheat only showed a wide range of variation between 25.4 and 28.7 grams. The highest value was registered for sample 3, while the lowest was sample 1. In contrast, no significant variations were found in the moisture, ash, lipid and carbohydrate values. However, samples 3 and 4, cultivated respectively in the Villanders and St. Leonard areas in the Bolzano region, had a higher protein content (over 13.3% d.m.) than other samples.

Table 1: Chemical composition of groats from Italian buckwheat samples.

Chemical composition	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1000 grains weight (g)	28.70	26.80	25.40	28.60	27.60
Moisture (%)	12.80	12.90	13.30	13.40	13.10
Protein (% d.m.)	11.00	11.70	13.60	13.10	12.60
Ash (% d.m.)	2.65	2.23	2.63	2.58	2.61
Fat (% d.m.)	3.35	2.93	3.37	3.37	3.34
Soluble carbohydrates (% d.m.)	63.70	64.90	61.00	61.40	61.60
Total dietary fibre (% d.m.)	6.48	5.27	6.08	6.14	6.25
Insoluble	5.29	4.13	5.21	5.26	5.17
Soluble	1.19	1.14	0.87	0.88	1.08
Percentage soluble fibre	18.40	21.60	14.30	14.30	17.30
Tannin	0.44	0.31	0.40	0.48	0.38

Table 2: Relative amount of protein fractions in Italian buckwheat samples (% dry matter).

Sample	Albumins	Globulins	Prolamins	Glutelins	Residues
Sample 1	18.36	44.16	0.69	22.73	14.06
Sample 2	18.29	44.30	0.71	22.15	14.58
Sample 3	18.41	44.29	0.84	22.56	13.90
Sample 4	18.15	44.18	0.85	22.48	14.34
Sample 5	18.80	44.36	0.59	22.71	13.54

Table 3: Aminoacid composition (g/100g of protein) of groats from Italian buckwheat samples.

Aminoacids	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Aspartic acid	10.38	9.64	9.68	10.0	9.78
Threonine	3.93	3.69	3.57	3.65	3.64
Serine	5.07	4.88	4.67	4.67	4.63
Glutamic acid	18.73	17.45	18.95	19.17	18.39
Proline	3.01	2.68	3.08	3.30	2.98
Glycine	6.33	5.92	5.87	5.89	6.06
Alanine	4.72	4.35	4.37	4.58	4.43
Cystine	2.67	2.58	2.70	2.50	2.70
Valine	5.7	5.03	5.29	5.35	5.42
Methionine	2.15	2.73	2.60	2.24	2.44
Isoleucine	4.09	3.73	3.81	3.84	3.77
Leucine	7.01	6.50	6.65	6.84	6.76
Tyrosine	2.98	2.65	2.88	3.08	2.69
Phenylalanine	4.79	4.73	4.68	4.59	4.60
Lysine	6.07	5.76	5.80	5.91	5.79
Histidine	2.56	2.36	2.40	2.47	2.49
Arginine	9.84	9.26	9.74	9.84	9.66

Total dietary fiber content ranged around 6% with the exception of sample 2 (5.3%). In particular, sample 1 showed the highest fiber content (6.5% in d.m.) of all samples set. The insoluble fraction, made up of lignin, cellulose and some hemicelluloses, which represents the main part of dietary fibre, reached in the same sample 5.3% in d.m. The soluble fraction (gums and mucilages) the importance of which is due to its chelating properties with metals, exceeded 20% of the total only in sample 2, while in samples 3 and 4 it was barely more than 14%.

The percentages of the protein fractions present in the buckwheat cultivated in Italy are given in Table 2. Almost half of the protein content in the five samples analysed was constituted of globulin with values of over 44%, while prolamins represented the smallest fraction (0.7%); the albumin (18%) and glutenin (22%) contents were constant in all the examined samples. The variations found in the protein fractions, even though minimal, are mainly attributed to climatic factors, different soil types, as well as the seed's ability to mature and harvest time.

Aminoacid profiles of the samples analysed are illustrated in Table 3. Unlike the proteins, the aminoacid content was virtually constant and all the samples had the characteristic of being high in lysine. The gels with the electrophoretic bands of the albumins present in the buckwheat samples are given in photographs 5 and 6. Proteins with molecular weights (Da) of 29,000, 45,000, 66,000, 97,400, 116,000 and 205,000 were used as standard. From the analysis of the gels it can be observed that no characteristic band can be individualized for each single variety and, at the same time, greater variations are found within a variety than among different varieties; this is mainly due to the undefined genetic characteristics of the various Italian buckwheat crops.

Figure 1: Flowering common buckwheat in the Bolzano region.

Figure 2: Common buckwheat field after harvest.

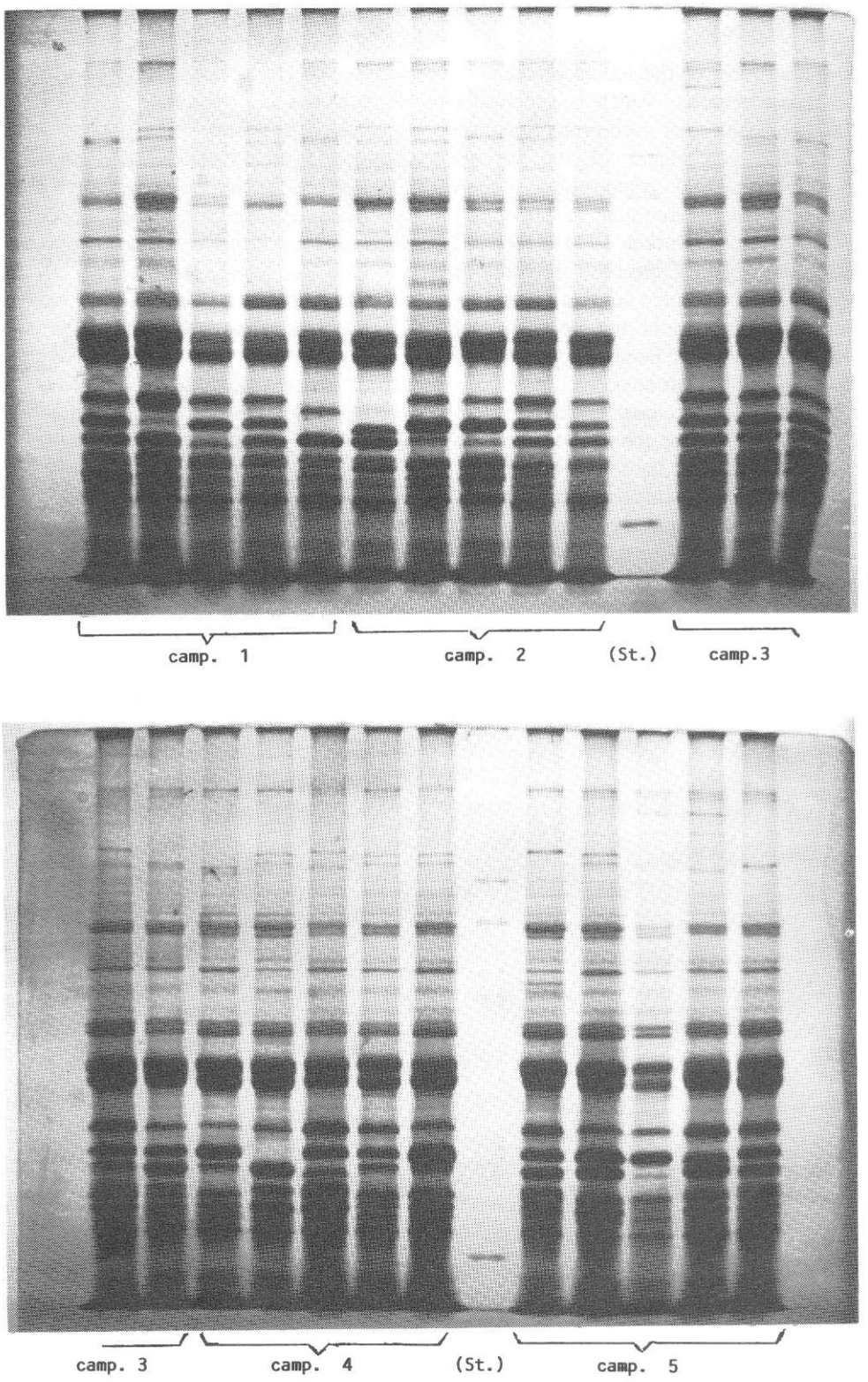

Figure 3: Electrophoretic bands of total proteins extracted from single seeds in five buckwheat samples from Italy (camp. 1-5 = samples 1-5; St. = standard).

Conclusion

The study shows that most of the Italian production, although there is not a variety which is genetically characteristic and constant, is of good protein quality, as regards both the percentages of the various fractions and the aminoacid composition. Italian samples are in this regard simmilar to previously studied buckwheat samples (Durkee 1970, Kreft and Javornik 1979, Javornik and Kreft 1980, Javornik et al. 1981). It should also be pointed out that the results show that single varieties could not be identified according to the electrophoretic bands, in view of the great variations found within varieties.

Moreover, it is possible to characterize the whole national production using other parameters, such as the high level of protein and/or the tannin content, as shown in the approximate chemical composition analyses carried out on the samples.

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Quantitative relationships between the growth and development of buckwheat and temperature and light

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Key words: *Fagopyrum esculentum*, *F. tataricum*, growth and development, quantitative relationship, temperature and light

Abstract

Quantitative relationships between the growth and development of buckwheat and temperature and light were analyzed. They clearly show that temperature and light can accelerate or delay the course of different growing periods. The optimum light for accelerating reproductive growth was found. The concept of light duration effect of the shortest and longest seedling-budding stage was defined. Based on these results, a preliminary classification of light responses of buckwheat varieties tested has been suggested.

Kvantitativni odnos med rastjo in razvojem ajde ter temperaturo in svetlobo

Analizirana so bila kvantitativna razmerja med rastjo in razvojem ajde ter temperaturo in svetlobo. Temperatura in svetloba lahko pospešita ali zavreta potek določenih stopenj razvoja rastlin. Ugotovili so optimalno točko za pospešitev reproduktivnega razvoja. Definirali so dolžino osvetlitve potrebno za počasen oziroma hiter razvoj do začetka cvetenja. Na osnovi rezultatov predlagajo predhodno opredelitev načina reagiranja rastlin ajde na vplive osvetlitve. (Prevod uredništva).

Introduction

Temperature and light are the main environmental factors which affect the speed of growth and development of crops and the characteristics of a variety. Buckwheat is widely distributed in China from the south to the north and from the easteren seacoast with an elevation of a few meters to the Qinghai-Xizang plateau with an elevation of thousands of meters. This indicates that buckwheat possesses a wide adaptability to high or low temperatures as well as to long or short light duration. Although it has been generally recognized that buckwheat is a temperature-loving and short day crop (Lin Rufa 1984, Ohnishi 1990), the quantitative relationships between the growth and development of buckwheat and temperature and light have been little analyzed. On the basis of the authors previous works (Hao Xiaoling 1989), the result of three years' tests was analyzed with respect to the effects of temperature and light and data of national ecological tests in buckwheat, and the present experiment was a further study on quantitative relations between the growth and development and temperature and light.

Results

1. Analysis of responses of different varieties and environmental effects on variations of growing period

The length of the growing period of buckwheat is affected by the hereditary features of the variety and environmental effects. As shown in Table 1, the variation coefficients caused by the environment are greater than those caused by the variety.

2. Accelerating and delaying reactions of temperature on the growth and development of buckwheat.

Analysis of the results of the seeding date test on common buckwheat 83-230 and Jiujiang tartary buckwheat, Table 2, indicates that the relationship between temperature (X) and the number of days from the emergence of the seedlings to the beginning of flowering gives an exponential function:

Common buckwheat 83-230, $Y = 41.13 * 0.9669^{X}$ ($r = 0.9995$)
Jiujiang tartary buckwheat, $Y = 50.91 * 0.9720^{X}$ ($r = 0.9997$)

Table 1: The number of days of growth and development for 10 buckwheat varieties sown in different areas.

Varieties	Changchun Jilin	Wuloumuqui	Yulin Shannxi	Baoding Hebei	Taiyuan Shanxi	Xining Quinhai	Yongsheng Yunnan	Weinig Guizhou	Jianyang Fujian	Environments Average	Variation range	CV%
Tartary Buckwheat												
Ba 18	89.0	93.0	65.0	92.0	113.0	123.0	78.0	82.0	77.0	90.2	65-123	20.1
Yimeng	94.0	--	90.0	108.0	112.0	108.0	86.0	99.0	78.0	96.9	78-112	12.4
Round seed	92.0	94.0	89.0	98.0	111.0	99.0	86.0	98.0	62.0	93.1	62-111	15.3
White	89.0	--	81.0	98.0	87.0	108.0	80.0	94.0	76.0	88.0	76- 99	9.8
Feng huang	88.0	68.0	76.0	88.0	83.0		86.0	94.0	75.0	85.1	68-108	13.8
Common Buckwheat												
Jian	74.0	89.0	61.0	68.0	67.0	102.0	52.0	68.0	56.0	70.8	52-102	22.4
83-230	82.0	81.0	59.0	73.0	63.0	59.0	66.0	69.0	63.0	72.6	59- 95	16.4
Daxilun	82.0	64.0	70.0	94.0	81.0	106.0	88.0	99.0	75.0	84.3	64- 99	16.3
Eqi	82.0	69.0	75.0	77.,0	--	106.0	81.0	94.0	59.0	80.4	59-106	18.0
Jing chuan	86.0	91.0	73.0	73.0	--	106.0	72.0	82.0	58.0	80.3	58-106	18.0
Varieties												
Average	85.8	81.1	73.9	86.9	89.6	106.1	77.6	87.9	67.9	--	--	16.6
Range	74-94	64-94	59-90	68-108	63-113	95-123	52-88	68.99	56-77	--	--	--
CV%	6.7	15.3	15.5	15.4	22.5	6.9	14.6	13.6	13.3	13.6	--	--

This shows that temperature has an obvious accelerating effect on the reproductive growth of buckwheat; as the temperature rises, the number of days from emergence of seedlings to early flowers decreases. Since buckwheat has an indeterminate inflorescence, within a certain temperature range it can fully utilize the natural light and heat energy. At later sowing dates, the average daytime temperature increases gradually, which is beneficial for the indeterminate inflorescences to grow fully. So the effective growing periods are prolonged and the total accumulated temperature in all stages increases. For example, common buckwheat and tartary buckwheat sowed on June 8 grew during the season of highest temperature, so the total accumulated temperature is the highest. After that, the average daytime temperature decreases gradually and the total accumulated temperature in all stages is reduced (Table 3, Figure 1).

This is an important biological reason for the wide adaptability of buckwheat in both area and seeding time. Thus, the effect of temperature on the growth and development of buckwheat is expressed quantitatively and results in great variations in buckwheat yields.

3. The regulating function of light duration on the growth and development course of buckwheat

Owing to the wide adaptability of buckwheat in its seeding time, light duration has a regulating effect on the growth and development course of buckwheat. It can be seen from Table 4 that in contrast to the accelerating effect of temperature, short light duration accelerates the reproductive growth of buckwheat. The number of days (Y) for seedling-budding stages and light duration hours (X) of four varieties is given by a quadratic equation, as shown in Table 5, and 8-10 hours is the most effective duration range for accelerating the seedling-budding stage. With under 8 hours of light, the accumulation of photosynthetic products and the vegetative growth of buckwheat are restrained. This restraint would be unfavourable for the transition of vegetative growth to reproductive growth. With over 10 hours, the long light duration delays the formation of the flower organs. To a certain extent, in conjunction with temperature, light affects the growth and development of buckwheat not only in quantity but also in quality. This is consistent with the analytical results reported by Cai Yan et al. (1989) regarding light tests on common buckwheat. The equations for eight varieties are shown in Table 6.

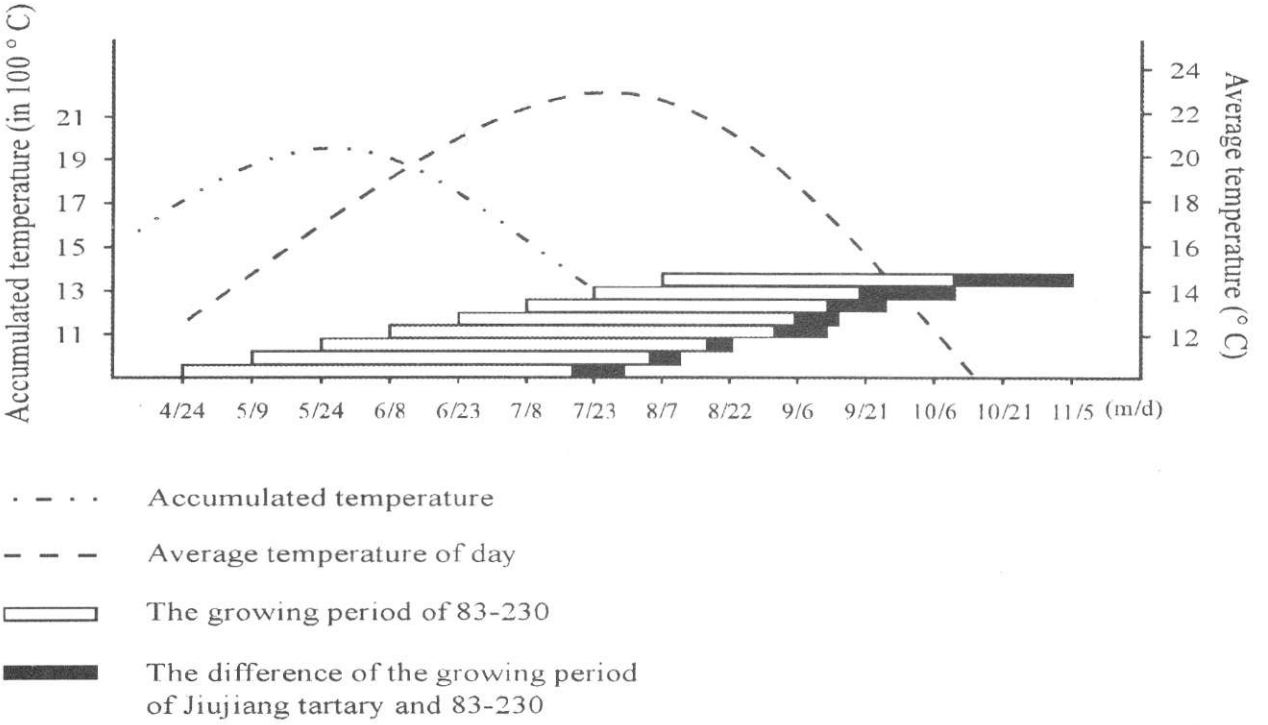

Figure 1: Total accumulated temperature (see Table 3) and average temperature in a day of common buckwheat 83-230, and Jiujiang tartary buckwheat under different sowing dates.

4. The shortest and the longest seedling-budding stage in buckwheat varieties

Analyzing the data of the light test reported by Cai Yan (Table 7) and according to the reactions of different varieties to light duration, the seedling-budding stage can be divided into short and long. The shortest needs the smallest number of days for the seedling-budding stage under optimum light duration conditions. The longest needs the most days under unsuitable light duration. As mentioned above, the responses of different varieties to light duration obviously differ. The difference between the shortest and the longest seedling-budding stage of a photosensitive variety is 42 days, while that of a photo-obtuse one is only 6 days. It is worth noting that this obvious difference appeared mainly among varieties which belong to the longest seedling-budding stage type, while varieties with the shortest are very close. They are all clustered within 10-11 days. This phenomenon indicates at least 3 points:

(1) The similarity of the shortest seedling-budding stage in varieties of buckwheat reflects their hereditary identity with reaction to light. The shortest seedling-budding stage may therefore be regarded as the basic starting point of the seedling-budding stage.

(2) The longest seedling-budding stage reflects the postponed ecological seedling-budding stage of buckwheat varieties in unsuitable light duration. It is really a variable measure of the ecological seedling-budding stage. The greater the difference, the more sensitive it is to short light duration. Thus its development demands a strictly short light period, and a narrower adaptable range.

(3) Analysis of the seedling-budding stage of buckwheat shows that this is the stage when reaction to light occurs. The light duration in this stage directly affects the time of the transition from vegetative growth to reproductive growth. Eight varieties tested can be classified into three types, according to the difference in the number of days of the seedling-budding stage of different varieties with different light durations.

A. Short light duration, very sensitive type. The difference in the number of days for the longest and the shortest seedling-budding stage is over 25 days. An example would be the Guixi common buckwheat.

B. Short light duration, sensitive type. The difference is 20-25 days, examples would be Hubei Nò. 3, Shengxian common buckwheat, and Pingli common buckwheat.

C. Short light duration, less sensitive type. Varieties whose difference is below 20 days all belong to this type.

Table 2: Number of days of growth and development of buckwheat at different sowing dates.

Variety	Sowing date (m/d)	Temperature (°C)	Emergence of seedling to begining of flowering (d)	Emergence of flowers to maturation (d)	Sowing to maturation (d)
Common	4/24	11.8	26	55	101
Buckwheat	5/ 6	14.6	25	56	100
83-230	5/24	17.7	23	58	98
	6/ 8	18.8	25	57	101
	6/23	23.5	22	50	90
	7/ 8	21.6	19	46	80
	7/23	24.3	16	39	66
	8/ 7	23.9	17	44	71
Tartary	4/24	11.8	39	49	122
Buckwheat	5/ 9	14.6	32	53	113
Jiujiang	5/24	17.7	30	53	109
	6/ 8	18.8	31	59	118
	6/23	23.5	25	55	108
	7/ 8	21.6	24	47	94
	7/23	24.3	26	45	95
	8/ 7	23.9	30	54	109

Table 3: Accumulated temperature of common buckwheat 83-230 and Jiujiang Tartary buckwheat on different sowing dates (°C).

Sowing date	Accumulated temperature Common Buckwheat 83-230			Accumulated temperature Jiujiang Tartary Buckwheat		
(m/d)	Emergence of seedling to begining of flowering	Emergence of flowers to maturation	All stages	Emergence of seedling to begining of flowering	Emergence of flowers to maturation	All stages
4/24	460.0	1168.6	1721.2	697.2	1128.5	1839.7
5/ 9	469.8	1295.6	1878.4	650.2	1233.4	1996.1
5/24	472.2	1339.0	1906.0	666.8	1205.1	1986.4
6/ 8	565.2	1267.3	1911.7	703.8	1274.7	2103.8
6/23	470.6	1086.9	1857.5	597.1	1151.6	1870.3
7/ 8	456.9	947.7	1493.5	622.4	893.9	1654.5
7/23	367.5	856.4	1328.7	570.5	736.7	1471.3
8/ 7	335.5	714.3	1146.3	587.9	674.0	1381.7

Discussion and conclusions

1. Reactions of buckwheat varieties to temperature changes show that buckwheat is generally a crop sensitive to positive accumulated temperature, but that sensitivities are different in different growth and devolopment stages. Before reproductive growth, a rise in temperature can shorten the seedling-budding stage and accelerate early flowering. After the beginning of flowering, because of the indeterminate inflorescence characteristics of buckwheat, with a rise in temperature, new inflorescences develop continually. Thus the growing period is prolonged, which results in the full use of the effective accumulated temperature. However, the total accumulated temperature as a whole increases. This is the fundamental difference between buckwheat and other crops in responding to accumulated temperature. This is also a biological characteristic which makes buckwheat a very adaptable and widely distributed crop.

2. Buckwheat is a crop characterized by growth and development which can be accelerated by short light duration. The relationship between light duration and the number of days of the seedling-budding stage is a negative quadratic curve. The optimum light duration is 8-10 hours. Under natural conditions, the number of days of the growing period, especially that of the seedling-budding stage, is clearly affected by the changing day length. This affect is mainly shown by the variations in the longest seedling-budding stage. Under unsuitable light conditions, the longest seedling-budding stage of a short light sensitive variety is long and vice-versa. We can therefore conclude that undoubtedly the seedling-budding stage is the light-sensitive stage of buckwheat i.e. the light stage of buckwheat.

3. The light dependent duration of the shortest seedling-budding stage can be regarded as a hereditary light duration dependence feature. The shortest seedling-budding stage of all varieties tested is at 10-11 hours light. The difference among varieties is very small. Cultivars of buckwheat can be classified into three types: short light duration, strongly sensitive; short light duration, sensitive; and short light duration, weak sensitive.

Table 4: The number of days of the seedling-budding stage of different varieties under different light duration.

Varieties	6 hours	10 hours	14 hours	24 hours	Average
83-230	24	23	25	31	25
Jiujiang tartary buckwheat	36	30	38	46	37
Yunnan round seed	60	47	52	84	60
Zhangjiacou black	49	46	48	98	60
Average	42.25	36.50	39.75	64.75	46.06

Table 5: Relationships between the number of days and light length of four varieties in the seedling-budding stage.

Varieties	Quadratic equation
Zhagjiacou black tartary	$y = 73.18 - 5.61 x + 0.28 x^2$
Yunnan round seed	$y = 86.86 - 6.21 x + 0.25 x^2$
Jiujiang tartary	$y = 45.15 - 2.24 x + 0.095 x^2$
83-230	$y = 25.75 - 0.51 x + 0.03 x^2$
Average	$y = 57.73 - 3.64 x + 0.16 x^2$

Table 6: Regressive analysis of the results of the light test of 8 common buckwheat varieties (from experiment results by Cay Yan et al. in 1988).

Varieties	Quadratic equation	Coefficient of correlation
Jingbian	$y = 25.85 - 3.15 x + 0.17 x^2$	0.905
Pingli	$y = 23.77 - 2.89 x + 0.17 x^2$	0.926
Weining	$y = 24.80 - 1.90 x + 0.066 x^2$	0.850
Lunan	$y = 21.29 - 1.28 x + 0.105 x^2$	0.896
Guixi	$y = 26.00 - 4.18 x + 0.289 x^2$	0.963
Hubei 3	$y = 22.27 - 2.40 x + 0.158 x^2$	0.887
Shengxian	$y = 25.60 - 3.33 x + 0.196 x^2$	0.946
Japan Mudan	$y = 18.08 - 1.87 x + 0.130 x^2$	0.928
Average	$y = 19.41 - 2.12 x + 0.14 x^2$	0.942

Table 7: Difference of the number of days from sowing to bud-emerging of different varieties under 6-12 hours light (from experiment results by Cai Yan et al. in 1988).

Varieties	The shortest number of days to bud-emerging (d)	The longest number of days to bud-emerging (d)	Difference of the shortest and the longest (d)	Average
Jingbian	10.2	26.9	16.7	16.7
Pingli	10.2	31.2	20.9	18.7
Weining	11.1	18.3	6.0	12.5
Lunan	11.3	24.5	13.2	17.5
Guixi	10.0	52.7	42.7	26.5
Hubei 3	10.0	33.2	23.2	21.1
Shengxian	10.7	33.3	22.6	19.6
Japan Mudan	10.9	28.8	17.9	17.6
Average	10.6	29.8	19.2	18.6

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Influence of planting date and variety on some characteristics of buckwheat plants in Eastern Croatia

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Key words: number of branches, number of leaves, plant height, planting date, seed yield, variety

Abstract

The influence of planting date upon some quantitative characteristics of different varieties of buckwheat plants (height, number of branches, number of leaves and seed yield) was investigated in the agroecological conditions of eastern Croatia. It was found that higher values of the investigated characteristics of all varieties of buckwheat were achieved by a June planting date than by a date in July. The highest yields were achieved with Darja and Petra varieties in an early planting date, and with Petra variety at a later date. Buckwheat cultivation as a stubble crop without irrigation depends on the distribution and quantity of precipitation in July and August.

Vpliv časa setve in kultivarja na nekatere lastnosti rastlin ajde v vzhodni Hrvaški

Avtorji so raziskovali vplive različnih rokov setve na lastnosti rastlin ajde (višina, število stranskih poganjkov, število listov in višina pridelka) v agroekoloških razmerah vzhodne Hrvaške. Višje vrednosti teh lastnosti so pri vseh proučevanih kultivarjih ugotovili za ranejši rok setve v juniju v primerjavi z julijskim rokom. Najbolj rodna sta bila pri prvem roku setve kultivarja Darja in Petra, pri kasnejši setvi pa Petra. Če ni namakanja, je pridelek ajde zelo odvisen od padavin v juliju in oktobru. (Prevod uredništva).

Introduction

Buckwheat is grown in the Republic of Croatia in comparatively small areas of both social and private property. Due to its short vegetation period it is grown mostly as a stubble crop after barley and wheat, or as a second crop. In the eastern continental part of Croatia, that is in Slavonia, buckwheat is efficiently grown on the lands of the Agricultural Farm Feričanci where it is sown every year in quite large plots after cereals. Extensive herbological investigations of buckwheat have already been carried out in this area (Knežević and Baketa 1989, 1992).

Successful stubble buckwheat cultivation in the investigated area depends on the right timing of planting because the summer months are known to have extended dry periods which are unfavourable for buckwheat in the phenophase of flowering. Robinson (1980), Gubbels and Campbell (1986) stressed the importance of planting dates after their extensive investigations of buckwheat in areas of Minnesota and Canada. The extent of buckwheat production also depends on the characteristics of individual varieties, i.e. on their reactions to specific agroecological conditions, according to reports by Kubiczek (1986), Alekseeva (1986) et al.

This work describes the results of two years investigations of the influence of planting dates on the yields of various buckwheat varieties in the agroecological conditions of eastern Croatia.

Material and Methods

The investigations were carried out in 1991 and 1992 on a pseudogley type of soil. In both years, buckwheat was sown in Feričanci after maize, in two planting dates. The first planting date was in June (June 5, 1991 and June 12, 1992), and the second date was in July (July 12, 1991 and July 17, 1992). The soil was prepared by standard techniques.

The test was laid by the split-plot method with four repetitions, in which the main factor (A) was the planting date, and subfactor (B) varieties of buckwheat. The plot size was 5 square meters. Buckwheat was sown by hand in rows with narrow spacing, i. e. with a 15 cm spacing between the rows, the amount of seeds being 100 kg/ha. The test included the following varieties: Darja, Petra, Darina, Rana 60 and Siva. Immediately after

planting, weed control was applied by means of the preemergent herbicide Dual 500 (metolachlor) 3 l/ha. The buckwheat was harvested by hand, and the seed yield is expressed in dt/ha. During these two years, investigations were carried out three times per season and involved the following morphological characteristics of the plants: height in cm, number of branches and number of leaves per plant. The seed yields were established in 1991 but were not considered in 1992 due to the lack of moisture.

Climatic conditions in the investigated years are shown in Figure 1.

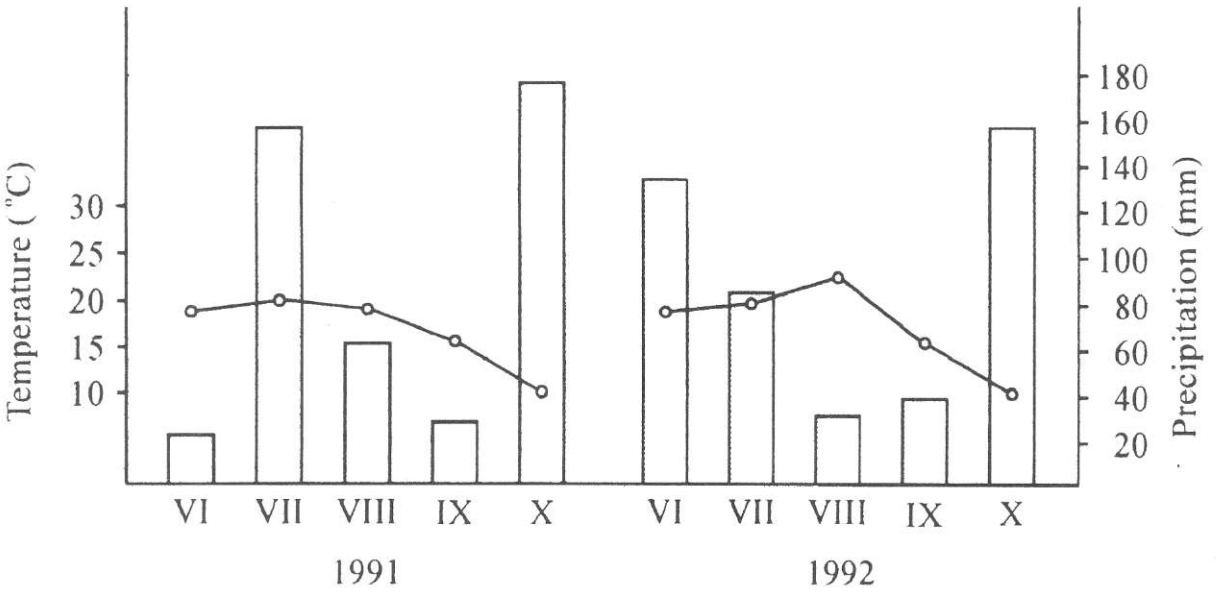

Figure 1: Mean monthly temperature (o----o) and the amount of precipitation (▭) in the vegetation period of buckwheat in the Feričanci region.

Results and Discussion

Plant height

Buckwheat height varied with respect to the years of investigation, the planting date and the varieties. In 1991, when the distribution of rainfall was relatively favourable, the variety Darja was founded to have the highest mean value in height (80.0 cm). The same variety showed much lower values (49.2 cm) in the drier year of 1992. The variety Darina had the lowest height in both years, with 71.0 cm in 1991 and 44.9 cm in 1992. The heights of other varieties averaged in both planting dates as follows: from 72.5 to 79.0 in the year with more favourable climatic conditions, and from 45.0 to 50.3 cm in the drier year (Table 1).

The differences in plant heights in relation to planting date in 1992 are highly significant ($P < 0.01$), while the differences in heights between the varieties are only significant for 1991 ($P < 0.05$). Interaction between the planting date and the variety is not statistically significant for plant heights (Table 2).

Number of branches

There were differences in the investigated years and the planting dates in relation to the ramification of the buckwheat plants, expressed by the number of branches per plant. In 1992, with the early planting date, an average number of 4.1 branches per plant was formed in all varieties, which was 20% higher than at the later date (Table 1). In the same year, the average number of branches varied from 3.1 to 4.3 per plant with both planting dates. The highest number of branches per plant was found with Darja and Rana 60 varieties. All varieties had smaller values for these characteristics in 1991. The reason is probably in the somewhat poorer density of plants in 1992, which resulted in a more favourable light and space balance among plants.

In terms of number of branches, statistically justified differences were determined between the planting dates and the varieties in 1992 (Table 2).

Number of leaves

The number of leaves as an indicator of photosyntethic potentials of buckwheat plants varied according to all the investigated factors. In 1991, the plants formed an average of 15.2 leaves per plant at both planting dates and in all varieties, and 14.1 leaves per plant in the drier year of 1992 (Table 1).

The variety Rana 60 developed the largest number of leaves, 16.3 per plant in the year with more rainfall. In the drier year the same variety reacted with a considerably decreased number of leaves, only 12.7 leaves per plant. Other varieties in the test also had a smaller number of leaves in that year, their average numbers ranging from 13.7 to 15.6 leaves per plant. The 1992 investigation results also show that there are statistically significant differences between the planting dates in relation to their characteristics. Varieties sown at the later date had an average of 12.7 leaves, which is 18% lower in comparison to the earlier sowing of varieties. Differences between the number of leaves by varieties, as well as the interaction between the planting date and variety were highly significant in 1992 (Table 2).

These results suggests the adaption of plants to longer dry periods by decreasing the surface of transpiration, i. e. the number of leaves.

Table 1: Influence of planting date and variety upon some quantitative characteristics of buckwheat plants (mean values and variability coefficient).

Varieties	Planting dates							
	June 5 1991		July 12 1991		June 5 1992		July 12 1992	
	Plant height in cm							
	$\bar{x}$	CV	$\bar{x}$	CV	$\bar{x}$	CV	$\bar{x}$	CV
Darja	77.0	16.2	83.0	14.8	61.3	15.2	37.1	19.7
Petra	77.0	16.1	78.0	14.9	59.9	14.0	40.7	21.4
Darina	76.0	19.7	66.0	19.8	55.9	17.2	33.8	20.1
Rana 60	75.0	16.7	70.0	17.0	56.6	16.8	35.2	15.9
Siva	82.0	11.1	74.0	19.9	52.8	12.9	37.2	19.1
	Number of branches per plant							
	$\bar{x}$	CV	$\bar{x}$	CV	$\bar{x}$	CV	$\bar{x}$	CV
Darja	2.7	48.1	2.8	17.9	4.4	38.6	4.3	18.6
Petra	3.2	37.5	2.6	23.1	3.4	44.1	2.7	44.4
Darina	3.6	44.4	2.8	14.3	3.9	41.0	3.8	21.1
Rana 60	2.9	48.3	2.9	13.8	5.3	28.3	3.3	21.2
Siva	2.9	48.3	2.8	17.9	3.7	40.5	3.1	38.7
	Number of leaves per plant							
	$\bar{x}$	CV	$\bar{x}$	CV	$\bar{x}$	CV	$\bar{x}$	CV
Darja	14.6	31.5	14.8	28.4	15.7	21.0	13.5	27.4
Petra	15.2	15.8	14.9	10.7	16.3	14.1	11.0	27.3
Darina	16.3	25.2	12.6	32.5	15.9	8.8	15.2	25.7
Rana 60	18.5	25.4	14.1	27.0	13.4	11.2	12.0	27.5
Siva	14.0	24.3	15.9	25.8	16.4	13.4	11.8	37.3

Seed yields

Buckwheat yields were measured in 1991 after the plants had had a sufficient number of vegetation days after each planting date. The vegetation periods for the early sowing (June 5) was 85 days for Rana 60 and Darina varieties, and 105 days for Darja, Petra and Siva varieties. At the later planting date, (July 12) all varieties had a vegetation period of only 68 days (till September 18), without any detrimental effect of frost.

The yields of all the investigated varieties differed significantly depending on the planting date (Table 3). The average seed yield of all varieties for the earlier sowing was 37.80 dt/ha, which was 22.7% higher than with later sowing. The established

differences are highly significant (Table 2). The varieties Darja and Petra gave the highest yields, 42.50 dt and 41.75 dt, whereas the variety Siva gave only 29.13 dt/ha. Buckwheat yields after the later planting date ranged from 11.75 dt to 16.88 dt/ha, and the greatest yield was from the variety Petra. The differences in seed yields between the investigated varieties are not statistically significant.

Due to lack of rainfall in 1992, yields were lower than 3 dt/ha for all investigated varieties and were not taken into consideration in data processing.

Buckwheat yield is higher in early planting than in later stubble planting, when it depends on the moisture condition in the soil.

Varieties react specifically to a lack of moisture in the soil. The variety Petra showed greater resistance to droughts, and when the soil had greater humidity this variety, along with Darja, gave greatest yields.

Acknowledgements

We would like to express our gratitude to Prof. dr. Ivan Kreft, Biotechnical Faculty, Agronomy Department, Ljubljana, Slovenia, for the seed materials that were used in these investigations.

Table 2: Statistical significance of investigated characteristics by year.

Factors of investigation	Planting date (A)	Variety (B)	Interaction A X B
1991			
seed yield (dt/ha)	**	ns	ns
plant height (cm)	ns	*	ns
number of branches / plant	ns	ns	ns
number of leaves / plant	ns	ns	*
1992			
plant height (cm)	**	ns	ns
number of branches / plant	*	*	ns
number of leaves / plant	*	**	**

* - $P < 0.05$

** - $P < 0.01$

ns - no-significant

Table 3: Influence of planting date and variety on seed yield of buckwheat (dt/ha).

	Planting date in the year 1991		
	June 5	July 12	$\bar{x}$
Darja	42.50	11.75	27.13
Petra	41.75	16.88	29.32
Darina	39.88	14.25	27.07
Rana 60	35.75	15.75	25.75
Siva	29.13	12.25	20.69
$\bar{x}$	37.80	14.18	25.99

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Effects of Harvade 25F on yield and quality of buckwheat seeds

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Key words: buckwheat, grain yield, Harvade 25F (dimetipin), regulation of development, Harvade 25F residues

Abstract

The influence of the synthetic growth regulator - Harvade 25F (active substance - dimetipin), on yield and certain quality features of buckwheat seeds, in pot and microfield conditions, was studied. Residues of this regulator in dehulled seeds and hulls were also investigated. In a microfield experiment, under stress caused by dryness, Harvade 25F acted profitably and distinctly on the yield of buckwheat seed. In a pot experiment, the preparate did not show any essential influence on the investigated parameters; although a slight increase in yield and the mineral content of seeds was noticed. The content of Harvade 25F residues in hulls was 10-15-times higher than in dehulled seeds in both experiments.

Vpliv Harvada 25F na pridelek in kakovost zrnja ajde

Raziskovan je bil vpliv sintetičnega rastnega regulatorja Harvade 25F (aktivna snov dimetipin) na pridelek in kakovost zrnja ajde v lončnih in pri poljskih poskusih. Raziskovani so bili tudi ostanki regulatorja v oluščenih semenih in v luskah. Pri poljskem poskusu je v razmerah suše regulator jasno vplival na pridelek ajde. Pri lončnih poskusih ni bilo bistvenega vpliva na raziskovane parametre; kazala pa se je rahla tendenca dviga pridelka in vsebnosti mineralnih snovi v zrnju. Pri obeh poskusih je bila vsebnost ostankov regulatorja v luskah 10 do 15 krat večja kot v oluščenem zrnju. (Prevod uredništva).

Introduction

Growing interest in buckwheat has recently been observed because of its high nutritional value (3, 10, 15). Buckwheat tops contain rutin, which is used in pharmaceuticals (7, 16). Buckwheat straw and hulls are fed to cattle and poultry (7, 11, 12) and has also high honey potential (7, 10). However, the grain yields of this crop are not high. Buckwheat is very sensitive to weather changes, because of its long flowering period (30-66 days), and non-uniformity in flowering of inflorescence, seed setting and ripening. This is the main cause of its low yields (1, 10, 11, 17). Some growth regulators may positively affect yield, seed setting, ripening and quality of this crop (5, 7, 8, 11). In this paper, the effect of Harvade 25F (active substance - dimetipin) on the yield and seed quality in pot and microfield experiments is reported. Harvade 25F residues in hulls and dehulled seeds were analyzed.

Material and methods

Pot and microfield experiments were carried out in 1992. Buckwheat plants were sprayed once with Harvade 25F (500 g dimetipin/ha) at the beginning of full ripening. In the pot experiment, Mitcherlich pots were used with an orchard soil and sand mixture. Plants were supplied with N - 2.4; P - 1.5; K - 2.0; Mg - 0.15 g/pot and microelements. There were 5 plants in each pot and soil water capacity was kept at 60%. Microplots experiment ($1m^2$) were performed on rye good soil and fertilized according to standards (9, 11). Plants were harvested at full ripening. Yield and yield structure components were analyzed, as well as protein and mineral composition (14). After dehulling, the residues of Harvade 25F were analyzed in hulls and dehulled nuts (4).

Results and discussion

In the pot experiment (Table 1) no significant differences were found in buckwheat yield, yield structure components, protein and microelements concentration after Harvade 25F treatment. Similar results with growth regulators were shown by Gubbels (2). In our experiment, we observed only a tendency to increase yield, thousand grain yield, and Ca and Zn content as a result of Harvade 25F treatment (Tables 1, 2).

Table 1: Pot experiment. Influence of Harvade 25F on the yield and some yield structure components of buckwheat (counted per plant).

Preparate	Weight of seeds (g)	Number of seeds	Weight of straw (g)	Mass of 1000 seeds
Control	18.5	753	29.2	24.6
Harvade 25F	19.1	769	29.8	25.8
$LSD_{0.05}$	-	-	-	-

Table 2: Pot experiment. Content of mineral components in buckwheat nuts after application of Harvade 25F.

Preparate	Protein	Mineral components						
	% N x 6.25	P	K	Ca	Mg	Cu	Mn	Zn
			(% d.m.)				(p.p.m.)	
Control	13.9	0.37	0.59	0.04	0.19	6.7	13.6	32.9
Harvade 25F	14.0	0.38	0.62	0.06	0.21	6.9	12.6	34.3
$LSD_{0.05}$	-	-	-	-	-	-	-	-

Li et al. (5) and Salnikov et al. (13) observed increases up to 26% of buckwheat yields in a similar experiment in spraying crops with growth regulators.

In the microfield experiment, the effect of Harvade 25F was pronounced. An increase of grain and straw yield was observed after Harvade treatment (Table 3). Other values were not significantly higher than the control (Table 4). Similar results were reported by Ciesielski et al. (1) after Harvade 25F treatment in field conditions. He obtained a 24% increase of buckwheat yield.

The obtained results (Table 3) suggest that Harvade 25F may be successfully used to increase buckwheat yield in field conditions. It is assumed that the increase may be due to the enhancement of flowering of higher row inflorescences and as a result of better supply of assimilates in the set grain. It may be due to better seed setting. Li et al. (5) found that growth regulators used by him increased photosynthesis and decreased internodes length in buckwheat.

It was further observed in this study that Harvade 25F residue content in both hulls and dehulled seeds was very low (Table 5).

The content of Harvade 25F in the hulls was found to be ten times higher. In the field experiment, the Harvade content was higher in both grain and hulls compared to the pot investigations.

Conclusions

1. An increase in buckwheat yield and yield structure components was found in microfield experiments after Harvade 25F treatment, probably because of dry stress conditions in 1992.
2. The quality of seeds was not affected by Harvade 25F.
3. Harvade 25F residues in buckwheat grain were low. A ten times higher content was found in the hulls in both pot and field experiments.

Table 3: Microfield experiment. Influence of Harvade 25F on yield and some yield structure components of buckwheat (counted per plant).

Preparate	Weight of seeds (g)	Number of seeds	Weight of straw (g)	Mass of 1000 seeds
Control	1.3	44	5.1	29.5
Harvade 25F	2.7	86	9.4	30.9
$LSD_{0.05}$	0.04	13.8	1.9	-

Table 4: Microfield experiment. Content of protein and mineral components in buckwheat seed after application of Harvade 25F.

Preparate	Protein	Mineral components						
	% N x 6.25	P	K	Ca	Mg	Cu	Mn	Zn
		(% d.m.)				(p.p.m.)		
Control	15.35	0.37	0.61	0.05	0.19	7.7	12.6	98.8
Harvade 25F	14.98	0.38	0.58	0.05	0.20	7.8	14.0	80.8
$LSD_{0.05}$	0.60	-	-	-	-	-	0.3	6.3

Table 5: Residue of Harvade 25F in hulls and dehulled seeds of buckwheat in pot and field experiments (in p.p.m.).

Preparate	Pot experiment		Field experiment	
	Dehulled seeds	Hulls	Dehulled seeds	Hulls
Control	0	0	0	0
Harvade 25F	0.003	0.027	0.024	0.364

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An investigation of the utility of buckwheat juice in the process of obtaining protein concentrates

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Key words: buckwheat juice, protein concentrates, proteolytic enzymes

Abstract

The juice obtained from lucerne plants was mixed with the juice obtained from buckwheat plants in a 1:4 volumetric ratio. In the raw material obtained in this way, the protein content increased slightly (in relation to lucerne juice), while the chlorophylls content and the proteolytic enzymes activity decreased. From the lucerne and the buckwheat juices and from their mixtures, the protein concentrates were precipitated by means of an anion Magnafloc LT-26 flocculant. The concentrate obtained from the buckwheat juice had a lower protein content than concentrate from the lucerne juice. In the concentrate flocculated from the mixture of juices, the protein level of both total and true proteins was close to the values obtained for the lucerne concentate. The highest precipitation efficiency was obtained for the buckwheat juice, though the quantity of the protein obtained from 1 dm^3 was the lowest.

Raziskava uporabnosti ajdovega soka za pridobivanje beljakovinskih koncentratov

S flokulantom Magnafloc LT-26 so bili dobljeni beljakovinski koncentrati iz sokov lucerne, ajde in njunih mešanic. Koncentrat iz ajde je imel manj beljakovin kot koncentrat iz lucerne. Koncentrat iz mešanice je imel vsebnost beljakovin blizu vrednosti za lucerno. Največji učinek precipitacije je bil pri ajdi, medtem ko je tu bilo najmanj beljakovin iz volumske enote soka. (Prevod uredništva).

Introduction

Fodder plants, with a rich protein content, are used in feeding polygastric animals. However, a high fibre content limits their usefulness in feeding monogastric animals. Separating proteins from the ballast of fibrin makes it possible to use the green parts of plants as an additional source of protein in feeding non-ruminants as well as people.

The process of regaining proteins from plants can be divided into three stages :

1. comminuting the plants
2. expressing juice from the obtained pulp
3. coagulating proteins contained in the green juice

During this process, two fractions are obtained: green juice and fibre residue. The fibre residue might be utilisable as a ruminant feed. The green juice contains the soluble protein. Several procedures have been recommended for preparing leaf protein concentrate /LPC/ from juice. The protein in the green juice can be coagulated by adjusting the pH to 3 - 4 (10), by application of heat or organic solvents (8, 11), or a combination heat and acid. The new trend in leaf protein concentrates is the application of high molecular weight polyelectrolyte to separate the protein from the green plant juice (3, 7, 8).

Leaf protein concentrates have a dry matter composition of between 50 and 60% true protein, 20 - 25% lipid and 10 - 15% carbohydrate, with the remainder being mainly ash. Thus this product has a slightly lower content of crude protein than fishmeal but a higher content than soya bean meal.

The traditional material for protein concentrate production is alfalfa. However, different species of plants (such as grass, clover, tobacco, lupine, ryegrass, rape and other) have been studied.

Buckwheat is a plant grown mainly for the seeds, but the fresh plant mass can be used in feeding monogastric animals by extracting the juice and then coagulating the proteins contained in it. There are very few reports on the direct application of buckwheat green fodder in feeding processes. The present paper shows the results of an unconventional use of the juice extracted from the biomass of this plant. Its utility in the process of obtaining protein concentrates as a factor inhibiting the activity of proteolytic enzymes of lucerne juice was investigated.

Materials and methods

The material for the study was green fodder of buckwheat and lucerne harvested before flowering from the experimental fields of RZD at Felin. The plants were preliminarily ground in a screw press and the pulp thus obtained then pressed in an expeller. The chloroplastic fraction of the proteins was precipitated with an anion flocculant Magnafloc LT - 26, using 0.4 g for 1 dm^3 of the juice. The concentratas were dried at 50°C. then ground and stored at a temperature of abount 4 - 8°C.

The total protein (N x 6.25) content was determined using the Kjeldahl method in a Kjel-Foss apparature, true protein was determined with 10% trichloroacetic acid, the chlorophyll content by the Arnon method (1), and the carotene and xanthophyll content using the Booth method (4). Total proteolytic activity was measured in 0.5 cm^3 of juice by adding 4 cm^3 of 5% casein in 0.1M phospate buffer (pH = 5.5) and incubated for 18 hours at 40°C.The unhydrolysed protein was precipitated with 4 cm^3 of 24% TCA and left at 4°C for 20 hours. The sediment was filtered off and the amount of protein in the obtained filtrate was determined by the Lowry method (9). Extinction was measured against the control in which hydrolysis was stopped with 24% TCA immediately after adding casein. The amount of protein was calculated from an analytical curve determined for various concentrations of tyrosine. Enzymatic activity was expressed in micromoles of tyrosine released by 1 cm^3 of juice in 1 hour.

Results and discussion

Due to the high content of lysine in the green matter, buckwheat seems to be an interesting material for obtaining protein concentrates from its juice. Investigations carried out earlier (2) confirm a sufficient protein content in the obtained preparations and composition from the point of view of feeding requirements. However, the quantites of the obtained preparations were small. In an analysis of the influence of conservants added to the buckwheat juice (5) on this chemical composition, very low values of proteolytic enzymes activity were obtained.

Flocculation of protein from the buckwheat juice gives quickly sedimenting deposits with good filtration properties. The authors of the present paper therefore carried out investigations on the utility of the juice in combination with lucerne juice (the raw material traditionally used for the production of concentrates) for the process of flocculation of protein preparations. In our investigations, the total protein content in the buckwheat juice was 19.23%, and the true protein was 81% (Table 1). The total level of chlorophylls was 3.02 mg/kg D.M., while the percentage share of chlorophyll "a" in the total content reached a value of 71%. The activity of proteolytic enzymes was 0.47 μmol/h of tyrosine released by 1 cm^3 of juice.

The juice obtained from lucerne was mixed with the buckwheat juice in a 1:4 volumetric ratio. In the raw material obtained in this way, the protein content increased slightly (in relation to lucerne juice), while the chlorophyll content and the proteolytic enzymes activity decreased.

Table 1: Chemical composition of plant juices.

	Lucerne juice	Buckwheat juice	Lucerne juice mixed with buckwheat juice
Dry matter (%)	9.25	4.86	8.34
Total protein (% D.M.)	37.62	19.23	38.94
True protein (% D.M.)	18.92	15.54	16.47
% of true protein in total protein	50.00	81.00	42.00
Chlorophyll "a" (mg/kg D.M.)	4.66	2.28	3.84
Chlorophyll "b" (mg/kg D.M.)	1.96	0.94	2.13
% of chlorophyll "a" in total chlorophylls	70.00	71.00	64.00
% of chlorophyll "b" in total chlorophylls	30.00	29.00	36.00
Activity of proteolytic enzymes (μmol/h of tyrosine liberated by 1 cm^3 of juice)	1.26	0.59	0.93

From the lucerne and the buckwheat juices and from their mixtures, the protein concentrates were precipitated by means of an anion Magnafloc LT-26 flocculant. The concentrate obtained from the buckwheat juice had a lower protein content than concentrate from the lucerne juice. In the concentrate flocculated from the mixture of juices, the protein level of both total and true proteins was close to the values obtained for the lucerne concentrate (Table 2).

The quantity of xantophylls remained unchanged, while the quantity of carotenes decreased.

The highest precipitation efficiency was obtained for the buckwheat juice (Table 3), though the quantity of the protein obtained from 1 dm^3 was the lowest. The addition of the buckwheat juice to lucerne improved the efficiencies of both the protein and dry matter, in comparison with concentrate flocculated from lucerne.

Table 2: Chemical composition of concentrates obtained from plant juice.

	Concentrate from lucerne juice	Concentrate from buckwheat juice	Concentrate from lucerne mixed with buckwheat juice
Total protein (% D.M.)	38.76	26.74	37.39
True protein (% D.M.)	24.21	13.32	25.12
% of true protein in total protein	63.00	50.00	67.00
Carotenes (mg/kg D.M.)	283.15	81.21	186.98
Xanthopylls (mg/kg D.M.)	384.66	177.37	373.97
% carotenes of total carotenoids	42.00	31.00	31.00
% xanthophylls of total carotenoids	58.00	69.00	67.00

Table 3: Yield of precipitation of dry matter and protein in concentrates obtained by fractionation of plant juice.

Specification	Protein g/dm^3	Protein^x	Dry matter g/dm^3	Dry matter^x
Buckwheat juice	9.34	100	49	100
Concentrate	6.69	72	25	51
Lucerne juice	34.80	700	93	100
Concentrate	14.30	41	38	41
Lucerne juice mixed with buckwheat juice	32.48	100	83	100
Concentrate	15.12	47	40	48

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Plant regeneration from mature cotyledons in a buckwheat (*Fagopyrum esculentum* Moench) germplasm collection

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Key words: *in vitro* culture, genetic variability

Abstract

Shoot regeneration from mature cotyledons was tested in 33 diploid and 4 tetraploid varieties belonging to the Slovenian buckwheat germplasm collection. Only 41% of the accessions formed shoots after 60 days of culture. Furthermore, in the responsive varieties, regeneration frequency was rather low, never exceeding 5% and often equal to 1-2%. Nevertheless, we demonstrated that both explant viability and shoot regeneration are strongly influenced by genotype and that a high variability can be found for these traits among and within buckwheat populations. The selection of a new variety characterized by increased levels of bud differentiation seems therefore feasible.

Regeneracija iz zrelih kotiledonov pri zbranih vzorcih ajde

Regeneracijo poganjkov iz zrelih kotiledonov ajde, smo testirali pri 33 diploidnih in 4 tetraploidnih vzorcih, ki so del slovenske genske banke. Po 60 dneh inokulacije je samo 41% proučevanih vzorcev formiralo poganjke. Frekvenca regeneracije je bila zelo nizka in ni presegla 5%, pogosto se je gibala od 1-2%. Kljub temu smo ugotovili, da sta vitalnost inokuliranih koščkov kotiledonov in regeneracija poganjkov močno odvisni od vzorca in njegove variabilnosti, ki smo jo našli med in znotraj populacij ajde. Zato smo selekcionirali ajdo z izboljšano diferenciacijo v primerjavi s testiranimi vzorci.

Introduction

A high frequency of plant regeneration is one of the essential prerequisites for the application of tissue culture in crop improvement. Since the earliest success obtained in tobacco and carrot, the list of plant species that can be used for tissue culture and regeneration has enlarged very much and now includes several major crops (Zryd 1988). In most species, progress was obtained through the choice of suitable genotypes followed by continuous technical refinement.

In buckwheat, plant differentiation has been achieved by culturing hypocotyls (Yamane 1974), mature cotyledons (Srejović and Nešković 1981), immature embryos (Nešković et al. 1987), anthers (Adachi et al. 1988, Bohanec et al. 1993), and even hypocotyl-derived protoplasts (Adachi et al. 1989, Lachmann and Adachi 1991, Gumerova 1991).

Especially with mature cotyledons and immature embryos, shoot differentiation was easily obtained; the most efficient protocol was characterized by a sequential change of hormones leading to cell commitment, bud formation, and eventually root production. This protocol was also used to insert the *NPT-II* gene into the plant genome, although from oncogenic strains of *Agrobacterium tumefaciens* (Miljuš-Djukić et al. 1992). In these experiments, only one or a small number of varieties was used, and the magnitude of genotypic effects on regeneration frequency was not determined.

In this article we provide information on the morphogenic competence shown by cotyledon fragments of several accessions belonging to the Slovenian buckwheat germplasm collection; we also demonstrate that characters such as explant necrosis and bud formation are strongly influenced by genotype.

Materials and Methods

Buckwheats

We tested 33 diploid and 4 tetraploid varieties of different origin (Table 1). The seed used for the trial derived from plants grown at different locations in different years. Seed was threshed beyond physiological maturity, dried to minimal moisture in a ventilated oven at 30°C, and stored at 4°C until use.

Sterilization and culture

Seed was surface-sterilized with ethanol 95% (1 min), dehulled without damaging the inner epidermis, and further sterilized in a commercial NaOCl bleach (13% active chlorine, 12 min) containing a few drops of Tween 20. The seed was then rinsed 6 times and dipped in sterile distilled water for 2 h. Pericarp, endosperm, and embryo axis were then removed and each folded cotyledon was cut in four 3-mm strips. Strips of each cotyledon were placed in 60-mm petri dishes containing 5 ml of culture medium. Mineral and organic solutions for cell commitment and bud formation were as described (Miljuš-Djukić et al. 1992); timing for substrate change was that recommended by Srejović and Nešković (1981). Rooting was obtained on a medium composed by MS mineral solutions (Murashige and Skoog 1962), B5 vitamins (Gamborg et al. 1968), 0.03 mg/l nickel chloride, 5 mg/l aurintricarboxylic acid, 0.5 indole-3-acetic acid, 0.25 mg/l indole-3-butyric acid, 10 g/l sucrose, pH 5.8. Substrates of any kind were gelified with 8 g/l Difco-Bacto agar. All cultures were grown in the light (34 $\mu E.m^{-2}.s^{-1}$ from Sylvania Gro-Lux, photoperiod 16/8 d/n) at 25 ± 0.5°C and subcultured every 3-4 weeks.

Data collected

The behaviour of a variety was determined on a 40-seed basis (i.e. 240 cotyledon strips). After 30 days of culture, explants were ranked in 6 classes according to their morphology. As bud formation was believed to occur also later than this date, we decided to describe the explants simply in terms of viability, presence of callus and roots. The number of regenerated shoots was measured after 60 days of culture and related to one hundred original strips, regardless of the presence of necrotic explants in the pool. To check the validity of the data, trials were repeated three times for cv. Darina.

To demonstrate that explant viability as well as regeneration of roots and shoots depend on genotype, we used single-plant progenies of cv. Darina. With regard to explant viability, we considered only those progenies in which early necrosis was relatively common and we acted similarly for shoot regeneration. As to root formation, we decided to carry out statistical analysis on 3 progenies characterized by a different capacity to develop roots from callus. For each character, we counted the number of explant series (i.e. seeds) in which it occurred at least once (or four times in the case of root production) and we compared this number with the value expected in case of random occurence; the latter was estimated through 1,000 computer simulations.

Hardening of regenerated plants

When plantlets had become vigorous and about 4 to 5 cm tall, they were potted in soil and covered with transparent plastic cups to maintain a high humidity. After two weeks, the cups were gradually opened until complete hardening was achieved. Plants were then transferred from the greenhouse into the open, where they flowered and set seed.

Results and Discussion

During the first 5 days, the cotyledon strips were cultured on a medium characterized by a high auxin to cytokinin ratio; exposure to 2,4-D caused cell dedifferentiation and division. At the end of this phase, explants had doubled or more than doubled their size, had become green (or red-green, due to the contemporary synthesis of anthocyanins), and in some instances had started callus formation.

After 30 days of culture, more than 90% of the explants had developed at least some callus (Table 1); exceptions to this rule were caused by early necrosis, to which accessions no. 16, 103 and Nagano, as well as cv. Bednja 4n, were particularly sensitive. Striking differences were noted in the capacity to form root initials; the two extremes were represented by cv. Darina and cv. Bednja 4n (approximately 77% of the explants with root producing callus *vs.* 5%) (Table 1).

Shoot regeneration was noted in 15 varieties but rates of regeneration varied a little (Table 2). Best results were achieved with accession no. 35 (from Korea) and 13 (from India). In this experiment, cultivars which were found responsive by Srejović and Nešković (1981), Nešković et al. (1987), and Miljuš-Djukić et al. (1992) regenerated a low number of shoots even if the pattern of explant growth and callus formation was quite similar to that described in the

Table 1: Explant viability, callus production, and root formation in a buckwheat germplasm collection after 30 days of culture (zero values omitted).

Variety	Origin	Frequency of explant type (%) (1)					
		1	2	3	4	5	6
cv. Siva	Slovenia					37.9	62.1
cv. Darja	Slovenia					38.9	61.1
cv. Darina	Slovenia		0.4		0.8	20.6	78.2
cv. Rana 60	Slovenia	0.7	3.6	0.7		70.0	25.0
cv. Hruszowska	Poland	0.7			0.7	46.8	51.8
cv. Černoplodna	Russia		1.3			58.3	40.4
cv. Šatilovska 5	Russia		0.6	0.6	0.6	46.8	51.4
cv. Botansoba	Japan		1.3		0.6	46.3	51.8
cv. Hashikami wase	Japan		2.5			67.5	30.0
cv. Ina zairai	Japan		1.9			58.1	40.0
cv. Shinanoishigo	Japan			5.6		38.1	56.3
cv. Kyushu akisoba	Japan					50.6	49.4
(#) cv. Bednja 4n	Slovenia			10.9		84.4	4.7
(#) cv. Petra	Slovenia		2.5			53.7	43.8
(#) cv. Pennquad	USA			1.3		55.3	43.4
(#) cv. Shinano oosoba	Japan					53.7	46.3
Črna I	Slovenia					60.8	46.3
Črna II	Slovenia					45.6	54.4
Črna III	Slovenia		0.6			26.3	73.1
Koroška črna	Slovenia				0.6	37.5	61.9
Sela na Krasu	Slovenia				1.9	40.6	57.5
Siva dolenjska	Slovenia		2.1			70.8	57.5
French grey	France		2.6			30.9	66.5
Lemadau	China			1.3	0.6	35.0	63.1
Wochuma	China					36.8	63.2
Tokyo	Japan		2.0	0.7		35.8	61.5
Nagano	Japan		13.9	0.7		44.3	41.1
type B	Italy		0.6			35.9	63.5
type C	Italy	0.6	2.5	0.6		38.5	57.8
type D	Italy		0.6			32.9	66.5
type E	Italy		1.3		0.7	39.5	58.5
type F	Italy					46.3	53.7
type 13	India					51.2	48.8
type 21	Hungary		0.7		0.7	51.4	47.2
type 35	Korea				1.2	27.5	71.3
type 16	The Netherlands	4.7	5.5		0.8	54.0	35.0
type 103	The Netherlands	1.6	10.9	5.5		22.6	59.4

(#) - tetraploid variety

(1) - explant type: dead 1. without callus and roots
2. with callus, without roots
3. with callus and roots
alive 4. without callus and roots
5. with callus, without roots
6. with callus and roots

standard protocol (Srejović and Nešković 1981). Attempts were made to find the reason for this, but neither hormone addition after autoclaving, nor the substitution of Difco-Bacto agar with four different Sigma agars (A-9799, A-7921, A-7002, A-4800), or gellan gum proved successful. Tests were made in two laboratories using different stock solutions of the reagents and different light sources. The possible

Table 2: Frequency of regeneration in a buckwheat germplasm collection after 60 days of culture.

Variety	Origin	Frequency of regeneration (%)
type 35	Korea	4.37
type 13	India	4.25
cv. Darja	Slovenia	2.86
Lemadau	China	2.50
Črna I	Slovenia	2.03
cv. Šatilovska 5	Russia	1.87
cv. Darina	Slovenia	1.56
cv. Siva	Slovenia	1.43
Črna III	Slovenia	1.28
Nagano	Japan	0.73
cv. Hruszowska	Poland	0.66
(#) cv. Pennquad	France	0.66
type E	Italy	0.66
type B	Italy	0.64
type F	Italy	0.62

(#) - tetraploid variety

effect of seed age was also taken into account. Since results did not vary, we concluded that the higher frequency of regeneration obtained by Nešković and co-workers was perhaps determined by light quality (4500 K Tesla lamps were not used in our trials) or, more feasibly, by factor(s) for which information was not given.

Other differences with respect to previous observations concerned shoot regeneration in root-producing calluses and flower differentiation in unrooted shoots. In our experiment, shoot regeneration occurred independently from the presence of roots in the explant (recent results obtained with hybrids specifically selected for high frequency of shoot regeneration confirm this evidence); in addition, flowering was observed in most unrooted shoots, provided that growth was vigorous.

It should be noted that explants derived from the same seed tended to behave similarly in relation to viability preservation, callus growth, and frequency of root formation. With regard to the former character, an analysis was made of 8 progenies with varying degrees of susceptibility to early necrosis (Table 3); the number of seeds from which necrotic explants developed was always significantly lower than that expected in the case of random occurrence of the trait (Table 3), indicating that genotype plays an essential role in determining cell viability under culture conditions. Shoot formation was also unevenly distributed across explants of different source (Table 4); the number of explant series in which the trait appeared was again significantly lower than expected (Table 4).

As in the germplasm collection, root production was the most variable character. Among progenies of cv. Darina, we could easily identify cases in which root production was present in 75% of the explants as well as cases in which it was relatively absent (20% or less). On the basis of this evidence, the important role played by genotype must be recognized. Nevertheless, the variability found within progeny was less pronounced than that observed for explant viability or shoot regeneration. In fact, in no instance was root production over-represented in explants of the same seed.

Conclusions

Only 41% of the accessions on trial formed shoots after 60 days of culture, which we consider a reasonable time for differentiation. In the responsive varieties, the frequency of regeneration was low, as was the number of shoots formed by a single regenerating explant. Therefore, in our experiments no variety readily suitable for biotechnological exploitation could be identified.

However, results suggested that factors which underlie culture efficiency such as explant viability and shoot regeneration are under genetic control and that a high variability can be found for these factors among and within buckwheat populations. Breeding for increased rates of regeneration seems therefore particularly promising.

Table 3: Effect of genotype on explant necrosis in eight progenies derived from cv. Darina.

Progeny	Necrotic explants (% on total)	No. of seeds involved	
		observed	expected (1)
1	24.4 a (2)	11	18
2	23.9 a	10	18
3	22.7 a	12	17
4	13.6 b	8	13
5	12.5 bc	9	13
6	11.9 bc	10	12
7	8.5 bc	8	12
8	6.8 c	6	9

(1) - in case of randomness and at a probability level higher than P = 0.05

(2) - means with a letter in common are not statistically different at P = 0.05, to compare progenies the chi-square method was used

Table 4: Effect of genotype on shoot regeneration in four progenies of cv. Darina.

Progeny	Explants with shoots (% on total)	No. of seeds involved	
		observed	expected (1)
9	13.6 a (2)	11	13
10	9.1 ab	8	9
11	7.4 ab	7	8
12	5.2 b	4	6

(1) - in case of randomness and at a probability level higher than P = 0.05

(2) - means with a letter in common are not statistically different at P = 0.05, to compare progenies the chi-square method was used

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Sixth International Symposium on Buckwheat

I. INVITATION

I am pleased to provide you with the registration materials for the VIth International Symposium on Buckwheat (VI ISB). The symposium will be held at the Faculty of Agriculture, Shinshu University, Ina, Nagano, Japan, 24-29 August 1995 under the auspices of the International Buckwheat Research Association (IBRA).

An ISB has been held every three years so far, in Slovenia, Japan, Poland, Russia and in China. It is a real pleasure and privilege for me to have the honor to host the second ISB to be held in Japan. More than one hundred scientists have taken part in each symposium, which has provided an impetus for international consultation among the participants.

Rice production in Japan in 1993 was reduced to less than 75% of the average yield because of very heavy cool weather damage. It is reported that we have never experienced so cool summer in this century, and that summer temperatures in 1993 were comparable to those in the Edo period (roughly 17-19th century), which several times caused terribly severe famine, with millions of people starving to death. We should again be aware that even when modern improved cultivation technologies and varieties are applied, yields can be severely reduced if environmental change occurs. Human beings are now confronted with global problems of an increase in world population, climatic change and pollution, creating great pressure on future agricultural production. Buckwheat, which can be cultivated under poorer conditions than other major crops, will play a more important roll in world crop production in the future.

Both grains and leaves of buckwheat are used as food. There are many ways of utilization, food processing and cooking in the world, which are all closely associated with local climates and cultures. Rutin and allergen in buckwheat are attracting the interest of medical scientists. Buckwheat has been a minor crop and a minor food from a global point of view, but increasing diversity of crops and foods are important for the future of human beings. I expect the VI ISB to be able to contribute to this through a large number of participants from diverse fields of research.

Nagano is a generally upland region and is a leading area of buckwheat production in Japan. Please do not hesitate to visit Ina through fear of the notoriously hot and humid summers in Japan. Nagano is famous for its summer resorts. The organizing committee invites you to spend time enjoying views of the beautiful mountains and buckwheat flowers, and buckwheat noodles and other Japanese traditional dishes. It gives me great pleasure to invite you to this symposium, which also offers you the opportunity to exchange the latest information on buckwheat research.

Toshiko Matano, Chairman
Organizing Committee
Sixth International Symposium on Buckwheat

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DEADLINES

PAPER MANUSCRIPT	FEBRUARY 28,1995
REGISTRATION	FEBRUARY 28,1995
HOTEL REQUEST FORM	FEBRUARY 28,1995
EXCURSION APPLICATION	FEBRUARY 28,1995

To those who wish to present a paper at symposium:

Please let us know as soon as possible the preliminary outline of your presentation (ie. on what subject, purpose, preliminary title) by sending us E-mail (tmatano@gipmc.shinshu-u.ac.jp; MHA01452@niftyserve.or.jp) or a computer diskette. We need the information to arrange the presentation program. If E-mail or computer system is not available, please let us know by a letter.

FEES

REGISTRATION FEE	by February 28, 1995	after March 1, 1995
PARTICIPANT	US$ 270	US$ 300
STUDENT	US$ 135	US$ 150
ACCOMPANYING ADULT	US$ 100	US$ 125

LODGING FEE	US$ 60-110 per person for a night's stay
EXCURSION FEE	US$ 100

II. ORGANIZATION

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- Ministry of Agriculture, Forestry and Fisheries
- The Crop Science Society of Japan
- The Japanese Society of Breeding
- The Genetic Society of Japan
- Japanese Society for Bioscience, Biotechnology, and Agrochemistry
- Japanese Society of Nutrition and Food Science
- Japanese Society for Food Science and Technology

III. SCIENTIFIC PROGRAM

1. List of Topics:The symposium will cover the following disciplines in oral presentations.

1) Morphology and taxonomy
2) Genetics and breeding
3) Genetic resources
4) Biotechnology
5) Culture and production
6) Growth and development
7) Ecology and physiology of productivity
8) Production technology
9) Physiology and plant nutrition
10) Harvesting
11) Post-harvest technology
12) Product quality
13) Product nutrition
14) Processing technology
15) Ethnobotany
16) IBPGR
17) Other

These divisions are preliminary and the final organization depends on the range of topics submitted by participants.

2. Official Language: The official language of the symposium will be English.

3. Forms of Presentation: Each participant is encouraged to present a paper(s). There will be two forms of presentation:

A) Invited Papers (Special Lectures)
Special lectures covering various topics on buckwheat will be presented by invited speakers. Invited speakers will have 40 minutes for their presentations and an additional 10 minutes for discussion. Themes and names of invited speakers will be given in the final announcement.

B) Contributed Papers
Presenters will have 20 minutes, including speech and discussion. Slide and overhead projectors will be available.

4. Schedule (preliminary)

Date	Morning	Afternoon	Evening
Aug.24 (Thurs.)		Registration	IBRA Committee Meeting
Aug. 25 (Fri.)	Opening Ceremony	Oral Presentation	Welcome Party
Aug. 26 (Sat.)	Oral Presentation	Oral Presentation	
Aug. 27 (Sun.)	Oral Presentation	Workshops IBPGR session	Closing Ceremony
Aug. 28 (Mon.)	Scientific Excursion		
Aug. 29 (Tues.)	Departure	Optional tour	

1) Arrival and Transportation: There is no international airport located near the symposium site, Ina. The new Tokyo International Airport (Narita Airport), which is about 400 kilometers from Ina, is the nearest and the most convenient one. A highway bus is available from Narita Airport to Tokyo and from Tokyo to Ina. Transportation from the airport to Ina takes about 5.5 hours and costs about 60 US dollars. The organizing committee recommends you to arrive at Narita Airport, because transportation from other airports is much more awkward and costly. We also recommend that you arrive at Narita Airport before 2:00 P.M. if possible, to ensure arrival in Ina on the same day. Accommodation in Narita or Tokyo is very expensive (at least US$ 70-100 per night). Staff of the organizing committee will welcome you and assist you with the bus transfer at Shinjuku in Tokyo.

Narita Airport → Shinjuku : Airport limousine bus (bound for Shinjuku); ab. 2 hr
Shinjuku → Ina : Highway bus (bound for Iida or Komagane); ab. 3.5 hr

We would advise you to book your flight early, because the number of persons arriving in and leaving Japan may increases towards the end of August. Further information about transportation will be provided in the third (final) announcement.

2) Symposium Venue: The VIth International Symposium on Buckwheat (VI ISB) will be held at the Faculty of Agriculture, Shinshu University, Ina, Nagano.

3) Registration at the Symposium: The registration desk at the symposium venue will be open from 10:00 a.m. on Thursday, Aug. 24.

4) Information Booth: A general information and travel booth will be located in the symposium venue during the symposium. A representative from the official Symposium travel agency will assist participants with travel schedules.

5) Exhibition: An exhibition of harvesting machines, buckwheat products and books on buckwheat is planned. A list with outlines of institutes for buckwheat study in Japan and many other countries will also be displayed.

6) Social Functions: A welcome party and closing ceremony will be held. All participants of the Symposium and accompanying persons are invited to both events.

7) Scientific Excursion: An overnight excursion will be arranged. The tour will be through the major buckwheat producing area of Nagano and will include visits to offices and research institutions. Views of the high mountain area known as the "Japanese Alps" will be possible. The fee, including accommodation and meals, is US$ 100 per person. Those who want to participate in the tour, please use the attached Excursion Request Form. As accommodation capacity is limited, applications will be accepted on a first-come-first-served basis. If your application is not accepted, we will inform you as soon as possible, and refund your fee at the registration desk at the symposium venue.

IV. REGISTRATION INFORMATION

1. Registration

Those who wish to participate in the VI ISB are requested to complete the registration form attached to this announcement and return it to the secretariat by February 28, 1995. Further information will be provided following registration. The third (final) announcement will be issued in June 1995.

2. Payment, Cancellation and Refund

1) Registration fees

Registration fees are US$ 270 for a participant, US$ 135 for a student and US$ 100 for an accompanying adult (non-participant: family member) if paid by February 28, 1995. Registration fees are US$ 300 for a participant, US$ 150 for a student and US$ 125 for an accompanying adult after March 1, 1995. Registration will deemed to be cancelled if the registration fee is not paid before June 1, 1995.

The registration fee for a participant and a student covers social functions, refreshments between sessions and a copy of the Symposium Proceedings.

2) Method of payment

Payment may be made by A) Bank transfer, B) Bank draft, C) International money order.

All payments should be remitted in US dollars and made payable to VI ISB.

3) Cancellation and Refund

Cancellation of registration will be permitted until June 30, with a US$ 100 cancellation fee plus the actual costs of remittance of the refund.

V. PAPER PREPARATION INFORMATION

1. Deadline for Manuscripts of Papers

The manuscript of presented papers should be submitted to the organizing committee by February 28, 1995.

2. Manuscripts of Papers for Proceedings

Manuscripts of presented papers prepared for publication in the Symposium Proceedings should not exceed 10 pages (typed in single space on A4-sheets), including tables and figures.

> Please submit camera-ready manuscripts typed in single space on one side of A4-sheets, with 2.5 cm margins on all sides.
> See sample for detailed instructions.

The organizing committee recommends that you submit your manuscripts on a disk, together with the above mentioned camera-ready manuscripts.

If you can submit your paper on a disk, please specify;

1. Computer [IBM, IBM compatible, Macintosh, etc.],
2. Operating system [MS-DOS, PC-DOS, MS-Windows, etc.],
3. Disk type [3.5" 720KB or 1.44MB, 5-1/4" 720KB or 1.2MB, etc.],
4. Software [Word Perfect, Microsoft Word for Windows, Excel, etc.].

> Please include on the disc the manuscript file in ASCII, in addition to the file processed with your word processing or other software, and make sure that the paper copy and the disk copy match.

3. Guidelines for Paper Preparation

Each paper should be headed by the title in capital letters followed by the names of authors, institution and address.

SAMPLE

STUDIES ON THE AGROECOLOGICAL CHARACTERISTICS OF BUCKWHEAT VARIETIES IN EUROPE AND JAPAN

Marek RUSZKOWSKY*, Ivan KREFT** and Toshiko MATANO***

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**Agronomy Department, Biotechnical Faculty, University of Ljubljana, Ljubljana, SLOVENIA

*** Faculty of Agriculture, Shinshu University, Ina, JAPAN

VI. ACCOMMODATION

The organizers will reserve a number of rooms at hotel(s). The rate is US$ 60-110 per night with breakfast. Transportation (bus service) between the hotel and the symposium venue will be provided. A taxi service is also available, which would cost about US$ 10-15.

Please inform the secretariat of your accommodation needs by using the attached Hotel Request Form.

VII. OTHER INFORMATION

The 2nd Asian Crop Science Conference (2nd ACSC) is to be held in Japan in 1995 just before our VI ISB. Hundreds of participants, mainly from East Asian countries, are expected to gather in Fukui city from Aug. 21-23. Fukui is located in the coastal area facing the Sea of Japan, while Ina is located in one of the highest mountain areas. We encourage you to enjoy both sea and mountain. A transportation service from Fukui to Ina is to be provided by the ACSC organizing committee. Those who are interested in the 2nd ACSC, please contact the ACSC secretariat.

ACSC secretariat
Laboratory of Crop Science,
Faculty of Agriculture, University of Tokyo,
Bunkyo-ku, Tokyo 113, Japan
Fax: +81-3-3815-5851

VIII. CORRESPONDENCE

All correspondence concerning the VI ISB, including presentations, registration and accommodation should be addressed to the VI ISB Organizing Committee.

REGISTRATION FORM (1)

Sixth International Buckwheat Symposium, 24-29 August 1995,
Shinshu University, Ina, Nagano, Japan

Deadline for registration: 28 Feb. 1995

One person per form. Please duplicate for additional registrations.

We are planning to provide a list of participants to encourage active conversation among the participants.
Please attach your photo and let us know your major research subjects.

Please type to make sure that your information is recorded correctly.

1. Personal information

Name _____
First name Middle name Family name

Title ☐ Prof. ☐ Dr. ☐ Mr. ☐ Ms.
Organization _____
Address _____
_____
Country _____
Phone _____ FAX _____
Field of Research (ex. Genetics) _____
Major Research Subject _____
(ex. Incompatibility, Interspecific hybrids, etc.)

photo
about 5 x 6 cm, not smaller than 3.5 x 3.5 cm please

REGISTRATION FORM (2)

2. Participation and presentation information

I will attend the symposium.	☐ yes	☐ no
I need a personal invitation letter.	☐ yes	☐ no
Presentation	☐ yes	☐ no
I am a corresponding author.	☐ yes	☐ no

Please type or write in block letters.

Author(s)		
Title of Presentation		
I need a slide projector	☐ yes	☐ no
I need an OHP	☐ yes	☐ no

If you have other presentation(s), please type author name and title of presentation in the same style as above in the space below.

REGISTRATION FORM (3)

3. Fees	Registration Fee	by February 28, 1995	No. of persons	after March 1, 1995	No. of persons	Fee US$
	participant	US$ 270/person		US$ 300/person		
	student	US$ 135/person		US$ 150/person		
	accompanying adult	US$ 100/person		US$ 125/person		
	Total	-		-		(A)

HOTEL & EXCURSION REQUEST FORM

Hotel reservation & fee (per night's stay with breakfast/person)

Date	No. of persons including accompanying persons	
Aug. 24	male	female
Aug. 25	male	female
Aug. 26	male	female
Aug. 27	male	female
Aug. 28	male	female
Aug. 29	male	female
Total	male	female

Hotel type	No. of persons to share	Fee US$
A	1	110
B	2	80
C	3	70
D	5	60

If you wish to have your reservation secured, please deposit US$ 100 specifying hotel type.

Hotel type =	Deposit US$ (B)

Excursion reservation		No. of persons	Fee US$
Excursion	US$ 100/ person		(C)

If you do not wish to join excursion, please complete with 0.

Grand total (A)+(B)+(C)= US$ _____

Please keep a copy of this sheet for correspondence purposes.

REGISTRATION FORM (4)

Method of payment

☐Bank transfer I have remitted the above total of US$ _____ on _____
Date

through _____ to the following account.

Name of bank: Citibank, N.A., Shinjuku Minamiguchi Branch
Name of account: VI ISB
Account No.: 92267009
Address: 1-1-7 Nishishinjuku, Shinjuku-ku, Tokyo 160

Please attach a copy of the receipt of the remittance to avoid any problems that may arise with the bank transfer.

☐ **Bank draft** Please make the bank draft payable to "VI ISB" and send it by registered mail together with registration forms.

☐ **International money order** Please make the money order payable to "VI ISB" and send it by registered mail together with registration forms.

Note 1) Please keep a copy of this sheet for correspondence purpose.
2) All remittance handling fees must be paid by the remitter.
3) If the remittance covers more than one person, please specify the names of each person in the column below

Remark column: