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Research paper

Seed-setting habit of a semidwarf common buckwheat line

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ABSTRACT

The seed-setting habit of a semidwarf common buckwheat (*Fagopyrum esculentum* Moench) line was evaluated in a field experiment under standard and high nitrogen levels. The 'semidwarf line' and 'Kitawasesoba' were used in the present study. The main stem length of the 'semidwarf line' was approximately two thirds of that of the 'Kitawasesoba'. Minimal differences were observed in the numbers of nodes on the main stem and primary branches of the 'semidwarf line' and 'Kitawasesoba' among genotypes and nitrogen levels. No drastic decline of seed yield by the shortening of the main stem length along with the introduction of dwarfness was observed in the 'semidwarf line' compared with 'Kitawasesoba'. The number of flower clusters per plant of the 'semidwarf line' in the high nitrogen plot tended to be greater than that of the 'semidwarf line' in the standard nitrogen plot and 'Kitawasesoba' in both nitrogen plots. This increase in flower clusters occurred on branches. The number of seeds of the 'semidwarf line' in the standard nitrogen plot was considerably lower than that of the 'Kitawasesoba' in both nitrogen plots. Similar tendencies were observed in the weights of seeds. The decline of seed number and weight of the 'semidwarf line' in the standard nitrogen plot was mainly observed in branches. From these results, it was found that the seed-set in the 'semidwarf line' can be improved by nitrogen fertilizer application. Furthermore, seed production on branches may play an important role in the high-yielding ability of semidwarf common buckwheat.

INTRODUCTION

Common buckwheat (*Fagopyrum esculentum* Moench) is widely cultivated, and its seeds are used in various food items, including cereal, noodles, tea, and bread. The yield of common buckwheat is generally affected by its seed-set, with a low seed-set resulting in a lower seed yield (Woo et al., 2016). Furthermore, lodging, i.e., the tendency of the stem to bend until the plant is lying horizontal, is one of the serious problems affecting buckwheat and resulting in a drastic decline of seed yield (Tetsuka and Morishita, 1999). Many typhoons occur during summer in Japan, leading to damage by lodging in common buckwheat cultivation. Shortening the plant height by developing a semidwarf cultivar can be useful in its improving the lodging resistance. In Tartary buckwheat (*F. tataricum* (L.) Gaertn.), the semidwarf cultivar 'Darumadattan' has already been developed by gamma ray-induced mutations (Ministry of Agriculture, Forestry and Fishers 2013). Darumadattan has been shown to have improved agronomic characteristics and strong lodging resistance (Morishita et al., 2010; Kasajima et al., 2012; 2013; 2014). On the other hand, few practical semidwarf cultivars of common buckwheat were available for a long time; however, dwarf and semidwarf genes in common buckwheat have been reported (Ohnishi and Nagakubo, 1982; Minami et al., 1999). Recently, Morishita et al. (2015) reported that novel semidwarf common buckwheat lines were developed from their breeding population in 2009. Compared with 'Kitawasesoba', a leading

cultivar in Hokkaido, northern Japan, the semidwarf common buckwheat line has a plant height that is approximately 60% and a seed yield that is almost the same as or slightly lower than that of non-dwarf common buckwheat. (Morishita et al., 2015). Therefore, the dwarfness of common buckwheat is considered to be practical for cultivation in Japan.

The improved lodging resistance in common buckwheat is known to be observed for determinate cultivars as well as semidwarf cultivars (Ohsawa, 2011). The determinate cultivar shows shortened plant height based on a determinate inflorescence. It has been demonstrated that seeds that develop on branches constitute a larger proportion of the total seed number (Funatsuki et al., 2000). Kasajima et al. (2016) pointed out that seed production from flowers on branches arising from the lower order nodes on the main stem plays an important role in increased seed yield of determinate common buckwheat. These reports suggest that the seed-setting habit in a semidwarf common buckwheat plant may differ from that of the normal cultivars based on the difference in morphological characteristics between the cultivars. An investigation of the seed-set from the viewpoint of morphological characteristics in semidwarf common buckwheat line is required. However, to date, there are no reports on the yield-determining process of semidwarf common buckwheat. The objective of the present study was therefore to clarify the seed-setting habit of semidwarf common buckwheat.

MATERIALS AND METHODS

Two common buckwheat genotypes were used in the present study. The 'semidwarf line' was discovered in 2009 after obtaining F_3 progeny through crossing between 'Horominori' and 'Mekei 20', and its semidwarf trait is controlled by one recessive gene (Morishita et al., 2015). 'Kitawasesoba' is the leading cultivar in Hokkaido, Japan (Inuyama et al., 1994). The cultivation of the materials used in the field experiment of the present study was conducted in the experimental field at the Memuro Upland Farming Research Station of the National Agriculture and Food Research Organization (NARO) Hokkaido Agricultural Research Center (Shinsei, Memuro, Kasai-Gun; longitude, 143° 03' E; latitude, 42° 55' N) from June to August, 2016. To evaluate the seed-setting habit, we designed standard and high nitrogen plots. In the standard nitrogen plot, a compound fertilizer, which comprised 1.8 g m⁻² of N, 7.2 g m⁻² of P₂O₅, and 4.2 g m⁻² of K₂O, was applied to the plots. In the high nitrogen fertilizer plot, ammonium sulfate containing 7.2 g m⁻² of N, equivalent to 5-fold that of N for the standard nitrogen plot, was applied in addition to the fertilizers used in the standard nitrogen plot. In both plots, fertilizer applications were performed only as a basal dressing. The seeds were sown in rows on June 3, 2016, with 60-cm row spacing and seeding density of 150 seeds m⁻². The experimental plot area was 7.2 m² (3 × 2.4 m). The plants of each plot were grown in a completely randomized design with two replications.

Ten plants in each plot were sampled on August 24, 2016 (maturity stage). The main stem length,

number of nodes on the main stem, and number of primary branches of the plants were investigated after the sampling. Subsequently, plant samples were air-dried over a period of two weeks. The number of flower clusters on main stem and each primary branch were then collected and counted. We investigated the seed number and weight on the main stem and branches after hand-threshing. In addition, the seed yield in each plot was investigated by unit area sampling.

RESULTS AND DISCUSSION

Table 1 shows the morphological characteristics and seed yield of the 'semidwarf line' and 'Kitawasesoba' grown under different nitrogen levels. The main stem length of the 'semidwarf line' was approximately two thirds of that of 'Kitawasesoba'. In both genotypes, the main stem length in the high nitrogen plot tended to be longer than that in the standard nitrogen plot. The difference in main stem length between the standard and high nitrogen levels was larger in the 'semidwarf line' than in 'Kitawasesoba'. However, minimal differences were observed in the number of nodes on the main stem and the number of primary branches of the 'semidwarf line' and 'Kitawasesoba' among genotypes and nitrogen levels. These results indicated that the decrease in the main stem length of the 'semidwarf line' can be dependent on the shortening of internode length, which was consistent with previous reports on semidwarf common buckwheat (Morishita et al., 2015) and Tartary buckwheat (Kasajima et al., 2012; 2013). No drastic decline in seed yield of the 'semidwarf line' by the shortening of the main stem

length along with the introduction of dwarfness was observed as compared with 'Kitawasesoba'. The seed yields of the 'semidwarf line' and 'Kitawasesoba' in the high nitrogen plot tended to be higher than those of the standard nitrogen plot. Morishita et al. (2015) reported that the seed yield of the 'semidwarf line' was almost the same or approximately 80% of that of 'Kitawasesoba'. Similarly, in the present experiment, the usefulness of the 'semidwarf line' was confirmed, and it was shown that its yield may be improved by nitrogen fertilizer application.

Fig. 1 shows the number of flower clusters on the main stem and on each branch (branches 1–4) of the 'semidwarf line' and 'Kitawasesoba' grown

under different nitrogen levels. The number of flower clusters per plant of the 'semidwarf line' in the high nitrogen plot tended to be greater than that of the 'semidwarf line' in the standard nitrogen plot and 'Kitawasesoba' in both nitrogen plots. This increase in the number of flower clusters was dependent on the increased number of flower clusters on branches, particularly branches 2 and 3. The results of the present study indicated that the application of nitrogen fertilizer promotes the development of flower clusters that develop on branches rather than on the main stem. Based on the present results, it is considered that the seed-set of the 'semidwarf line' used in the present study was susceptible to nitrogen fertilizer application.

Table 1. Morphological characteristics and seed yield of a 'semidwarf line' and 'Kitawasesoba' grown under different nitrogen levels.

| | | Main stem length (cm) | Number of nodes on main stem | Number of primary branches | Seed yield (kg a ⁻¹) |
|----------------|-------------------|--------------------------|------------------------------------|-------------------------------|-------------------------------------|
| Semidwarf line | Standard nitrogen | 70.5 ± 3.4 | 13.7 ± 0.4 | 3.0 ± 0.1 | 16.3 ± 0.6 |
| | High nitrogen | 86.6 ± 4.1 | 14.9 ± 0.6 | 3.2 ± 0.2 | 20.0 ± 1.2 |
| Kitawasesoba | Standard nitrogen | 111.4 ± 2.7 | 12.4 ± 0.4 | 3.2 ± 0.2 | 19.4 ± 0.9 |
| | High nitrogen | 118.7 ± 2.9 | 12.9 ± 0.4 | 3.3 ± 0.2 | 22.3 ± 1.8 |

Data are shown as mean ± standard error of two replicates.

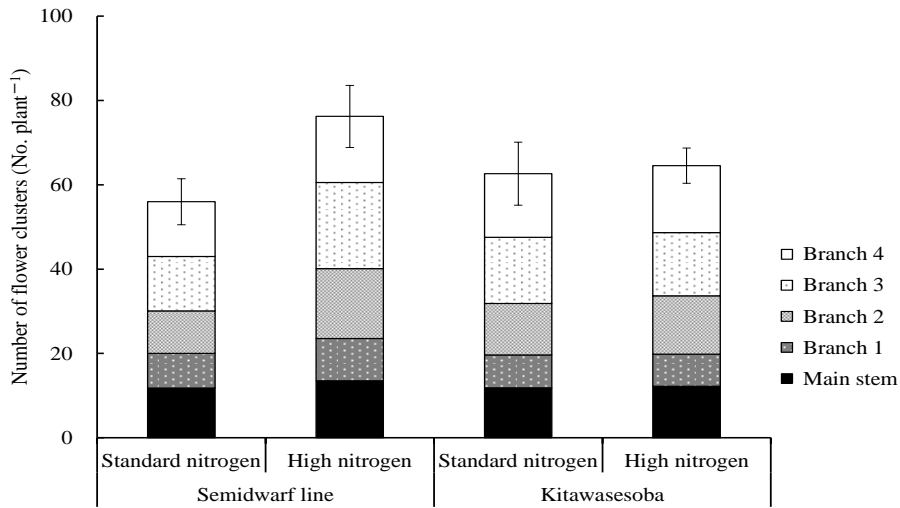


Fig. 1. Number of flower clusters in each part of a 'semidwarf line' and 'Kitawasesoba' grown under different nitrogen levels. Vertical bars represent standard errors of total flower clusters based on two replicates. The branches were numbered from the soil surface (branch 1) to the terminal part of the plant (branch 4).

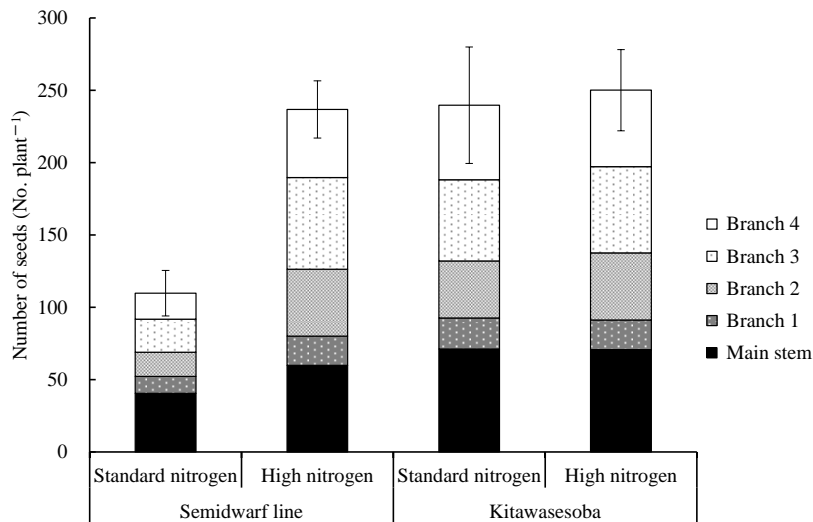


Fig. 2. Number of seeds on the main stem and on each branch (branches 1–4) of a 'semidwarf line' and 'Kitawasesoba' grown under different nitrogen levels. Vertical bars represent standard errors of total seed number based on two replicates. The branches were numbered from the soil surface (branch 1) to terminal part of the plant (branch 4).

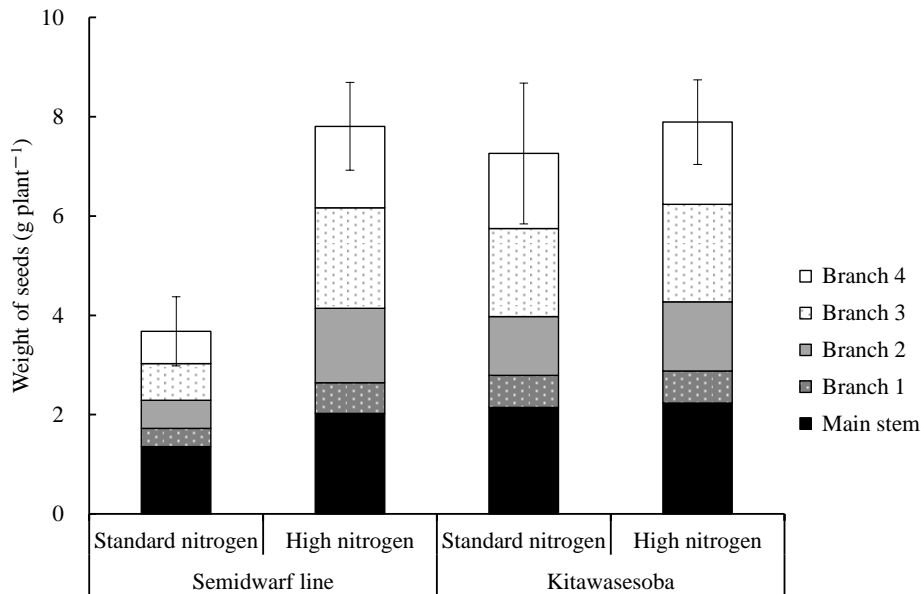


Fig. 3. Weight of seeds on the main stem and on each branch (branches 1–4) of a ‘semidwarf line’ and ‘Kitawasesoba’ grown under different nitrogen levels. Vertical bars represent standard errors of total seed weight based on two replicates. The branches were numbered from the soil surface (branch 1) to terminal part of the plant (branch 4).

Fig. 2 shows the number of seeds on the main stem and on each branch (branches 1–4) of the ‘semidwarf line’ and ‘Kitawasesoba’ grown under different nitrogen levels. The number of seeds of the ‘semidwarf line’ in the standard nitrogen plot was considerably lower than that of ‘Kitawasesoba’ in both nitrogen plots. On the other hand, the seed number of the ‘semidwarf line’ in the high nitrogen plot was almost the same as that of ‘Kitawasesoba’ in both nitrogen plots. Similar tendencies in regards to the weights of seeds were observed in the ‘semidwarf line’ (Fig. 3). The decline of seed number and weight of the ‘semidwarf line’ in the standard nitrogen plot was mainly observed in branches, particularly branches 2–4, rather than in

the main stem. These results indicated that the application of nitrogen fertilizer may improve the seed-set on branches of the ‘semidwarf line’ used in the present study. Furthermore, we clarified that the seeds that developed on branches play an important role in the yield-determining process of semidwarf common buckwheat.

In conclusion, it was found that the seed-set in a ‘semidwarf line’ can be improved by application of nitrogen fertilizer. Furthermore, the seed production on branches may play an important role in the high-yielding ability of semidwarf common buckwheat. In the future, we plan to carefully examine the responses of lodging in the ‘semidwarf line’ to heavy fertilizer application. The semidwarf

cultivar of Tartary buckwheat was found to have a higher rooting ability compared with the cultivar of standard height (Kasajima et al., 2015). Further studies will be required to examine the lodging resistance of semidwarf common buckwheat from the viewpoint of rooting ability.

Acknowledgements

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Research paper

Tartary, but not common, buckwheat inhibits α -glucosidase activity: its nutritional implications.

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Tartary buckwheat, α -glucosidase, diabetes mellitus

ABSTRACT

This study shows the presence of α -glucosidase inhibitor in Tartary buckwheat. The present study shows that standard Tartary buckwheat flour exhibited a high level of α -glucosidase inhibitory activity, whereas newly-developed Manten-kirari var., which exhibited a very low level of rutin-degrading enzyme, exhibited no α -glucosidase inhibitory activity. On the other, no α -glucosidase inhibitory activity was found in common buckwheat flour. Nutritional implications of these findings in view of diabetes mellitus prevention were discussed.

INTRODUCTION

Life-style diseases such as diabetes mellitus are a current, major nutritional problem globally. Prevention from these diseases is a subject of much interest in nutrition science. Much attention in food components, which, if any, might be effective for preventing from such life-style diseases, will be paid. Buckwheat (*Fagopyrum* spp.) is an important crop in many countries of the world (Ikeda, 2002; Kreft et al., 2003). There is a large variety of buckwheat products globally. There are two species of cultivated buckwheat, i.e., common buckwheat and Tartary buckwheat. R. Lin and his group (1992) showed that Tartary buckwheat, but not common buckwheat, lowers blood sugars in patients suffering from diabetes mellitus and that Tartary buckwheat flour also lowers serum lipid in patients suffering from hyperlipidemia (Lin et al., 1992). However, the exact mechanisms responsible for the observed beneficial effects for prevention of diabetes mellitus and hyperlipidemia remains uncertain. α -Glucosidase is a major enzyme responsible for the

gastrointestinal digestion of saccharides into glucose. α -Glucosidase inhibitor is used as drug curing diabetes mellitus. Although there are many factors involved in prevention from diabetes mellitus, α -glucosidase inhibitors in foods, if any, might inhibit the intestinal absorption of glucose, so maybe leading to the prevention of diabetes mellitus. In this connection, increasing attention in polyphenols present in red wine and various plant foods for beneficial effects in human health is currently paid (Renaud and De Lorgeril, 1992). It is well known that buckwheat, especially Tartary buckwheat, contains a high level of polyphenols such as rutin and quercetin. Polyphenols in common and Tartary buckwheat may have profoundly-beneficial effects on human health.

This study was undertaken to identify α -glucosidase inhibitor in Tartary buckwheat in view of clarifying a factor responsible for the report by Lin's group showing that the intake of Tartary buckwheat lowered blood sugar in patients suffering diabetes mellitus.

MATERIAL AND METHODS

Material

Two different types of Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) were used in this study. One type of Tartary buckwheat, harvested in China, was used

and was abbreviated as the standard Tartary buckwheat. Another type of Tartary buckwheat (var. Manten-Hikari) (HARC, 2017), which was bred by the group of Hokkaido Agriculture Research Center, was obtained. Since this Tartary buckwheat seed (var. Manten-kirari Tartary

buckwheat) has less or substantially no rutin-degrading enzyme (HARC, 2017). Therefore, this Tartary buckwheat contains a high level of rutin but lower level of quercetin. Common buckwheat (*Fagopyrum esculentum* Moench, var. Hashikami-wase), was used in this study. These buckwheat seeds were separately milled with a Brabender Quadromat milling machine with 231 μm sieving. Each flour obtained was subjected for analysis. Rice flour and wheat flour used were commercial products. Catechin and tannic acid, which were obtained from Nakalai Tesque Co., Kyoto, Japan, were of analytical grade.

Extraction

Buckwheat flour samples were extracted with 80% methanol for 1 h at room temperature with rotating. After extraction,

Statistical analysis

Statistical analysis was conducted using a personal computer with the program Excel (Microsoft Co., USA).

the suspensions were centrifuged at 10,000 rpm for 10 min. After centrifugation, the supernatants obtained were subjected for analysis.

Enzyme assay

α -Glucosidase assay was performed with maltose as the substrate according to the method described (Bergmeyer, 1974). Glucose released from the enzyme assay was analyzed with a commercial enzyme assay kit with glucose oxidase plus peroxidase. Inhibition assay for α -glucosidase was determined as follows: sample solutions tested were pre-incubated with enzyme solutions for 10 min at 37°C. After pre-incubation, remaining enzyme assay was determined with the substrate at 37°C for 30 min. Inhibitor constants (K_i) were estimated by the Lineweaver-Burk plot.

RESULTS AND DISCUSSION

Inhibitory activity against α -Glucosidase by methanol extract of Tartary buckwheat flour

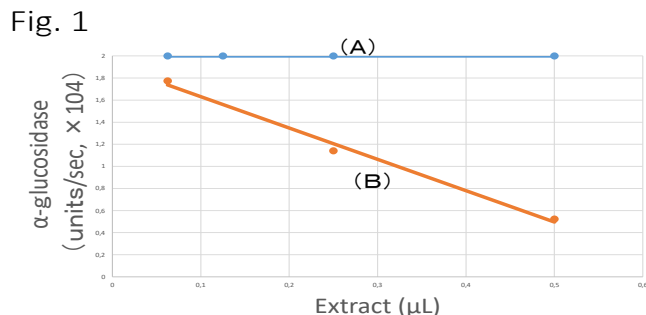


Fig. 1 Effect of Tartary and common buckwheat extracts on the activity of α -glucosidase. (A), common buckwheat extract; and (B), Tartary buckwheat extract.

Figure 1 shows the effects of methanol extracts of common and Tartary buckwheat flours. As shown in Fig. 1, the methanol extract of Tartary buckwheat exhibited inhibitory activity against α -Glucosidase, whereas the methanol extract of common buckwheat exhibited less inhibitory activity against this enzyme. The effect of heating for inhibitory activity against α -glucosidase by the methanol extract of Tartary buckwheat flour was examined. Heating did not

influence the inhibitory activity by the methanol extract of Tartary buckwheat flour (data not shown). This finding shows that the observed inhibitory activity against α -glucosidase by the methanol extract of Tartary buckwheat flour may be due to heat-stable components such as polyphenols present in buckwheat, but not to heat-unstable proteinaceous components.

Inhibitory activity of various polyphenols

It is well known that common and Tartary buckwheats contain various kinds of polyphenols such as rutin and quercetin. We tried to assay some polyphenols against α -glucosidase. Table 1 shows the

inhibition constant, as the Lineweaver-Burk plot method, of some polyphenolics against α -glucosidase activity. As shown in Table 1, quercetin exhibited extremely high inhibitory activity with very low inhibition

constant, so indicating that quercetin may be a strong inhibitor against α -glucosidase. Tannic acid also exhibited inhibitory activity against α -glucosidase. On the other hand, no inhibitory activity against this enzyme

was found with rutin and catechin. The observed inhibitory behavior (Table 1) with quercetin and rutin was very important as discussed below.

Table 1 Inhibition constant of various polyphenols against α -glucosidase

| Components | Inhibition constant ¹⁾ |
|-------------|-----------------------------------|
| Quercetin | 1.68×10^{-6} M |
| Tannic acid | 4.34×10^{-7} M |
| Rutin | No inhibition ²⁾ |
| Catechin | No inhibition ²⁾ |

1) Inhibition constant was estimated by the Lineweaver-Burk plot method.

2) The word “no inhibition” means that no inhibition against α -glucosidase was found under the assay conditions used.

No inhibitory activity against α -glucosidase by the Manten-kirari Tartary buckwheat

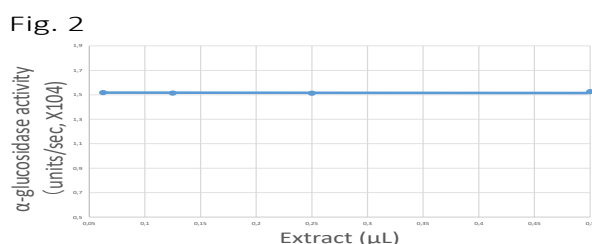


Fig. 2 Effect of Tartary buckwheat var. Manten-kirari extracts on the activity of α -glucosidase.

Figure 2 shows the effect of the Manten-kirari Tartary buckwheat extract against α -glucosidase. No inhibitory activity against α -glucosidase was found with the Manten-

kirari Tartary buckwheat. The Manten-kirari Tartary buckwheat exhibits no rutin-degrading enzyme. Therefore this Tartary buckwheat contains a high level of rutin,

which exhibited inhibitory activity α -glucosidase (Table 1), and contains less or no quercetin which have inhibitory activity (Table I).

α -Glucosidase is a major enzyme responsible for the gastrointestinal digestion of saccharides into glucose. α -Glucosidase inhibitor is used as a medicine curing diabetes mellitus through inhibition of intestinal absorption of ingested glucose. Although there are many factors involved in

the prevention from diabetes mellitus, α -glucosidase inhibitors in foods, if any, might inhibit the intestinal absorption of glucose, so maybe leading to the prevention of diabetes mellitus.

This study concludes that quercetin may be a factor responsible for the report by Lin's group showing that the intake of Tartary buckwheat lowered blood sugar of patients suffering diabetes mellitus.

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Liubov Kalynivna Taranenko

In loving memory of a plant breeder, teacher, expert

On September 12, 2017, at the seventy-ninth year of life, the heart of **Liubov Kalynivna Taranenko** stopped beating. She was a person filled with love and compassion for the surrounding world, an outstanding scientist, teacher, talented organizer, philanthropist, doctor of biological sciences, professor, active member of the Vavilov Society of Geneticists and Breeders of Ukraine, Director General of Research and Production Small Enterprise “Antaria” LLC.

Liubov Kalynivna was born on November 5, 1938, in Holokhvasty village of Volochyskiy district of Khmelnytsky region. In 1955, she graduated from the secondary school in Volochyskiy district of Khmelnytsky region. In 1960, she graduated from Kamianets-Podilskiy

Agricultural Institute (Agronomy Department).

From 1960 to 1966 she was an agronomist, a lead specialist in the Seed Agronomy Service of Pochayiv and Borshchivsky districts of Ternopil region. From 1967 to 1969 she was a postgraduate student majoring in breeding and seed production.

In 1970 she received a degree of a PhD in Agricultural sciences, having defended her thesis paper on the topic “Impact of pollinic regime on kernel percentage and seed productivity of tetraploid buckwheat” in specialty “breeding and seed production”.

From 1966 to 2016 she worked at the National Scientific Centre “Institute of Agriculture of the National Academy of Agricultural Sciences”, holding positions of a junior research scientist, senior research scientist, and from 1985 to 2013 she was the Head of the Cereal Crops Breeding Department.

The main direction of her research was the development of scientific principles of buckwheat breeding related to genetics of quantitative features of reproductive system components – a method for overcoming the species self-incompatibility, creation of inbred species and application of hybrid vigor effect during crop breeding. Liubov Kalynivna Taranenko developed principles, techniques and methods of buckwheat breeding for creation of the high-quality varieties of this crop.

For the first time, a unique method for overcoming interspecies incompatibility of buckwheat with the purpose of introgression of valuable features of wild species into a crop (the method is protected by the patent) was developed.

She made a significant contribution to the expansion of buckwheat polymorphism. The development of buckwheat genome improvement as a crop with unlimited growth processes is also unique, and is aimed at changing buckwheat architectonic, improving ratio of features and phases of its morphogenesis, thus ensuring high crop yields even in stressful conditions.

At the same time (1993-1998) she was the Head of Genetics, Breeding and Seed Production Department of Kyiv National Agrarian University. She created a scientific school in the field of “breeding and seed production”. Under the supervision of Liubov Kalynivna Taranenko, seven candidate theses in specialty 0601.05 – breeding and seed production - were defended.

The varieties of buckwheat created by Liubov Kalynivna Taranenko are used on more than 55% of buckwheat crop acreage of Ukraine (Astra, Kyivska, Lileia, Ukrainka, Antaria, Oranta, Sin-3/02, Olha, Nadiyna, Malva, Sofia and Volia). Some of these varieties are widespread abroad: buckwheat variety Oranta is entered into the Register of Plant Varieties in Poland, in Azerbaijan – variety Antaria, in Germany – variety Sofia.

After studying international practices, in 1992 she founded the first privately-owned breeding and seed production company “Antaria” in Ukraine, which reproduces high quality original and elite seeds of the most promising varieties of grain and cereal crops. She is the author of 128 scientific papers and 8 books. She delivered reports at international congresses and academic conferences in Bulgaria, Poland, China, Japan, Czech Republic, Russia, Korea and other countries. For many years she was a member of specialized councils for defense of thesis for a doctor’s degree at the National Agrarian University, the National Scientific Centre “Institute of Agriculture of the National Academy of Agricultural Sciences” and the Institute of Biological Fuel and Sugar Beets of the National Academy of Agricultural Sciences; of the commission on state awards at the National Academy of Sciences of Ukraine; was a member of IBRA international organization, etc.

In 2017, she initiated the creation and became the co-founder of the International Buckwheat Association in Ukraine.

For lasting personal contribution and outstanding achievements in the agricultural sector, she was awarded with certificates of honor of the Cabinet of Ministers of Ukraine, the Ministry of Agrarian Policy and Food of Ukraine, with the Order of Princess Olga (3rd class), international awards and IBRA medals.

The credo of this radiant and many-sided person was “I have devoted all my life to people, and there is nothing more valuable than my native land, freedom and bread in my land”.

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