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Front page photo: Experimental site at Stopar family ecological farm “Mežner” in Javorje (1180 m above sea level), near Črna na Koroškem, Slovenia. At the left Tartary buckwheat plants, right common buckwheat plants (See paper of Golob et al., this issue, page 15).

FAGOPYRUM volume 38(1) (2021)

CONTENTS

ORIGINAL PAPERS

INFORMATION

Fagopyrum 38 (1): 5-13 (2021)

Review paper

Recent advances in the nutritional, functional, and agronomic traits of Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.)

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ABSTRACT

Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) is considered a functional food because its seeds contain higher amounts of polyphenols (e.g., rutin) compared to common buckwheat. However, because of its highly bitter taste and difficulties in cultivation, the agricultural production and usage of Tartary buckwheat in food products remain limited. The nutritional and functional ingredients of Tartary buckwheat include quercetin, which causes its bitterness and is generated by rutinosidase (rutin-degrading enzyme). A nonbitter Tartary buckwheat variety with trace levels of rutinosidase has recently been developed. Despite such research, there is still a lack of agronomic information on Tartary buckwheat. Lodging can be a significant problem during its cultivation, and a lodging-resistant, semidwarf variety has been developed. This paper summarizes recent advances in our knowledge regarding the nutritional and agronomic traits of Tartary buckwheat. The information extends our understanding of the health benefits of Tartary buckwheat and the solutions to challenges in its agricultural production.

INTRODUCTION

Common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.) comprise the two species of buckwheat. Common buckwheat is cultivated throughout the world, mostly in Russia and China, whereas Tartary buckwheat production is limited mostly to China, Bhutan, and Nepal. Although limited in its production, Tartary buckwheat has several advantages compared to common buckwheat. Being an autogamous plant, Tartary buckwheat produces high and stable yields due to its high seed set. Its seeds contain rutin, a major polyphenol, at levels approximately 100 times more than that found in common buckwheat seeds (Kitabayashi et al. 1995a, b). It can be grown in cold and harsh climatic conditions, and it is recognized as a functional food. Thus, like common buckwheat, Tartary buckwheat has recently been attracting interest in several countries and regions, including Japan and Europe, because of its health benefits (Ikeda et al. 2012).

Despite its positive attributes, Tartary buckwheat cultivation and utilization in food products remain limited because of two main reasons. Firstly, the flour of Tartary buckwheat is highly bitter because of the presence of high concentrations of flavonoids (Fabjan et al. 2003). This is the main reason that Tartary buckwheat has never become a major food crop in many countries. Secondly, its cultivation is difficult because of its tendency to lodge due to its height (Hagiwara et al. 1999). To enable the mechanized and efficient cultivation of this plant, it is necessary to first address these key challenges. Recent research on Tartary buckwheat in Japan has focused on the development of novel varieties. For example, bitterness and lodging have been addressed by the development of nonbitter (Suzuki et al. 2014b) and semidwarf varieties (Shimizu et al. 2020). Both of these varieties have been evaluated in detail.

The nutritional and functional aspects of Tartary buckwheat have been reviewed previously (Ruan et al. 2020; Zhu, 2016; Kreft et al. 2020), but there is still a lack of information on its agronomic characteristics. This review focuses on presenting some new findings on the nutritional qualities, functionality, and agronomic traits of Tartary buckwheat.

Nutritional ingredients in Tartary buckwheat

Table 1 shows the general compositions of Tartary buckwheat, common buckwheat, and wheat flour. The data on Tartary buckwheat are from 'Manten-Kirari,' a leading variety in Japan. The main nutritional components of Tartary buckwheat do not differ significantly from those of common buckwheat, although their levels vary greatly by region, variety, cultivation, and milling methods. On the basis of data presented in Table 1, the

Table 1. *General ingredients in Tartary buckwheat, common buckwheat, and common wheat flours. Values are expressed in units per 100 g of flour.*

	Tartary buckwheat (Manten-kirari) (Buckwheat flour)	Common buckwheat (Buckwheat flour)	Common wheat (medium-strength flour, first grade)
Energy (kcal)	347	361	367
Water (g)	14.8	13.5	14.0
Proteins (g)	9.1	12.0	9.0
Lipids (g)	2.3	3.1	1.6
Carbohydrates (g)	72.5	69.6	75.1
Ash (g)	1.3	1.8	0.4
Sodium (mg)	-	2	1
Potassium (mg)	441	410	100
Calcium (mg)	17.1	17	17
Magnesium (mg)	204	190	18
Phosphorus (mg)	419	400	64
Iron (mg)	3.42	2.8	0.5
Zinc (mg)	3.22	2.4	0.5

The data for 'Manten-Kirari' were provided by the Japan Food Research Laboratories. The rest of the data were obtained from the Japan 2015 (7th edition) Standard Tables of Food Composition. '–' indicates 'no data.'

flours of common and Tartary buckwheat contain about 12% and 9% protein, respectively. Other reports suggest that the protein contents of the flour from both species do not differ significantly (Bonafaccia et al. 2003b; Qin et al. 2010). However, among the flours of 21 Chinese Tartary buckwheat genotypes, protein contents ranged from 6.82% to 15.02% (Qin et al. 2010), suggesting large genetic variations. Analyses of seeds from Tartary and common buckwheat show that the protein contents of the bran are twice those of the flour in both species (Bonafaccia et al. 2003b). Furthermore, the proteins of Tartary and common buckwheat share the same amino acid composition (Bonafaccia et al. 2003b; Qin et al. 2010). Lysine, an essential amino acid, occurs at high levels in albumins and globulins (Javornik and Kreft, 1984). Thus, the protein content in both the species of buckwheat is well balanced in terms of essential amino acid composition (Kreft et al., 2020). Moreover, Tartary buckwheat contains higher levels of the proteins soluble in dilute acids or bases, and those soluble in ethanol-water mixtures compared to those in common buckwheat (Ikeda et al. 2003).

The mineral composition of Tartary buckwheat has been studied by Bonafaccia et al. (2003a), Huang et al. (2014), and Ikeda et al. (2004). Both common and Tartary buckwheat have much higher potassium and magnesium levels than wheat (Table 1). Moreover, Tartary buckwheat has higher levels of iron; zinc; vitamins B1, B2, and B6; and total B vitamins than common buckwheat (Bonafaccia et al. 2003b). However, both species of buckwheat share similar compositions of dietary fiber (Bonafaccia et al. 2003b).

The functionality of Tartary buckwheat

Tartary buckwheat seeds contain higher levels of the functional nutrient rutin than common buckwheat. Rutin is a kind of polyphenol that exists widely in the plant kingdom, although among cereal crops, it is found only in buckwheat. It has antioxidant (Morishita et al. 2007) and antihypertensive (Matsubara et al. 1985) activities. Tartary buckwheat seeds contain about 100 times more rutin than common buckwheat. Specifically, 100 g of common and Tartary buckwheat seeds contains about 10–30 and 1100–2000 mg rutin, respectively, with large differences among varieties (Kitabayashi et al. 1995a, b; Morishita et al. 2006, 2007; Suzuki et al. 2020). Noda et al. (2020) reported that the rutin content of Tartary buckwheat bran is fivefold that of its flour, and the authors provide the proper roasting time and temperature for retaining these high levels of rutin in bran. Thus, our current knowledge on rutin in Tartary buckwheat seeds indicates that future studies should explore the possibilities of effective utilization of rutin in both flour and bran. The function of rutin in Tartary buckwheat is to protect the plant body from the environmental stresses of alpine regions, such as ultraviolet (UV-B) rays, low temperatures, and dryness (Kreft et al. 2003; Suzuki et al. 2005).

Tartary buckwheat also degrades and eliminates rutin in the flour, which happens when water is added, such as during noodle making. Rutin is a flavonoid glycoside that is degraded to quercetin and rutinose by the rutin-degrading enzyme rutinosidase, which is abundant in Tartary buckwheat flour and activated by water (Suzuki et al. 2002; Yasuda and Nakagawa, 1994). Quercetin is one of the causes for the strong bitterness of Tartary buckwheat. However, rutinosidase in flour can be deactivated by heat treatment at >70°C (Kawakami et al. 1995; Yasuda et al. 1992).

Meanwhile, the Hokkaido Agricultural Research Center of the National Agriculture and Food Research Organization of Japan has developed a Tartary buckwheat variety that contains high levels of rutin while keeping its bitterness low (Suzuki et al. 2014a, b). This was accomplished by first developing a method to detect rutinosidase on gels that involved staining of a copper–rutin complex. This led to the discovery of 'f3g162,' a Tartary buckwheat line selected from about 500 genetic resources and mutant lines for its low rutinosidase activity. The authors crossed 'f3g162' with 'Hokkai T8,' a Hokkaido standard variety, and selected 'Mekei T27' for its excellent agronomic characteristics and low rutinosidase activity, which is controlled by a recessive single gene (rutA) (Suzuki et al. 2014a). After further improvements in the variety's agronomic characteristics, such as plant height, yield, and maturity, it was registered and named 'Manten-Kirari' in 2014 (Suzuki et al. 2014b). The rutinosidase activity of 'Manten-Kirari' is extremely weak, which is a few hundredths that of the conventional variety 'Hokkai T8,' and the rutin content remains mostly stable even after processing the flour into noodles. Moreover, the antioxidant capacities of the noodles and cookies made from the flour of 'Manten-Kirari' are high (Ishiguro et al. 2016), and these foods are effective in reducing body fat percentage, body weight, and BMI (Nishimura et al. 2016). In recognition of their 'epoch-making variety,' the breeders of 'Manten-Kirari' were awarded the 2019 Japanese Society of Breeding Award.

Agronomic traits of Tartary buckwheat

In Japan, Suzuki et al. (2014b) reported grain yields of 'Manten-Kirari' ranging from 2.16 to 2.48 t ha^{-1} (Suzuki et al. 2014). In China and Italy, grain yields of 1.2–1.5 t ha^{-1} (Zhang et al. 2008; Xiang et al. 2016) and 2.29 t ha^{-1} (Brunori et al. 2006), respectively, have been reported. Thus, the potential yield of Tartary buckwheat appears to be around 2 t ha^{-1}. At actual production sites, however, the yields of Tartary and common buckwheat are often lower because of preharvest shattering and losses due to threshing and aborting by combined harvesters (Funatsuki et al. 2000; Morishita and Suzuki, 2017). Furthermore, Matsuura et al. (2005a, b) reported that Tartary buckwheat is more susceptible to excess soil moisture and salinity than common buckwheat. However, only a few studies have examined the effects of environmental stress on the growth and yield of Tartary buckwheat.

Kasajima et al. (2012a) compared the changes in main stem length, number of leaves on the main stem, number of primary branches, and SPAD values of common and Tartary buckwheat grown in Hokkaido, the northernmost region of Japan (Fig. 1). During the early

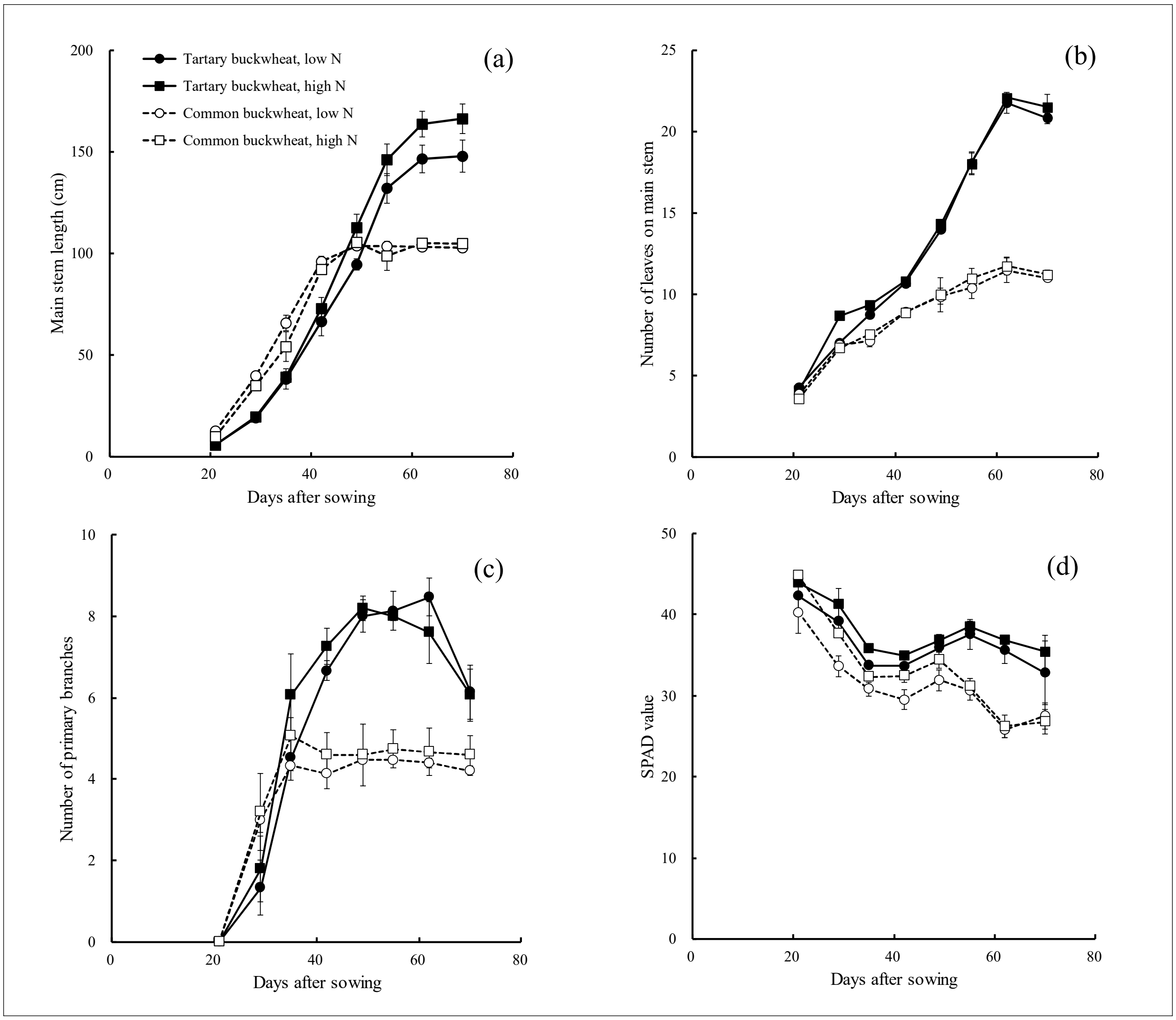

Fig. 1. *Changes in (a) main stem length, (b) number of leaves on the main stem, (c) number of primary branches, and (d) SPAD values in common buckwheat cv. 'Kitawasesoba' and Tartary buckwheat cv. 'Hokkai T8' under different nitrogen levels (Kasajima et al. 2012a). The plants were grown in an experimental field at Abashiri, Hokkaido, northernmost region of Japan. Low and high nitrogen levels correspond to 2 and 5 g m^{-2} of nitrogen as a basal fertilizer, respectively. Vertical bars represent standard errors.*

stage of growth, the main stem of common buckwheat is slightly longer than that of Tartary buckwheat, but the rate of stem elongation of common buckwheat plateaus at around 50 days after sowing, and the stem of Tartary buckwheat continues to grow rapidly. The main stem of Tartary buckwheat is about 50 cm longer than that of common buckwheat at maturity (Fig. 1a). Similarly, Tartary buckwheat has more leaves on its main stem and more primary branches than common buckwheat, and the differences in these parameters tend to be large (Fig. 1b, c). The SPAD value of Tartary buckwheat generally exceeds that of common buckwheat (Fig. 1d). At harvest, the Tartary buckwheat plant is taller and has more branches and leaves than the common buckwheat plant (Campbell, 2003; Morishita et al. 2006; Kasajima et al. 2012a).

Semidwarf Tartary buckwheat

The growth characteristics of Tartary buckwheat make it susceptible to lodging, i.e., the tendency of the stem to bend until the plant is lying horizontal, and it is a significant problem in the cultivation of Tartary buckwheat (Hagiwara et al. 1999). The lodging resistance of Tartary buckwheat may be enhanced by controlling the planting density or altering certain stem characteristics such as the lignin content and the activities of lignin-related enzymes (Xiang et al. 2016, 2019). Another approach is to shorten the plant, i.e., developing a lodging-resistant semidwarf cultivar. Dwarf and semidwarf genes have been reported in common buckwheat (Ohnishi and Nagakubo, 1982; Minami et al. 1999; Morishita et al, 2015), but little information regarding these genes is available in Tartary buckwheat genetic resources. Thus, seven semidwarf Tartary buckwheat mutants were developed by mutation breeding, resulting in the identification of two semidwarf genes, namely, *sdA* and *sdB* (Morishita et al. 2010). Subsequently, gamma-ray irradiation was used to develop the semidwarf variety, 'Darumadattan,' which was registered in 2013 (Shimizu et al. 2020). The height of 'Darumadattan' is almost half that of a standard Tartary buckwheat variety (Shimizu et al. 2020; Kasajima et al. 2012b). As shown in Fig. 2, the decreased height of 'Darumadattan' (previously known as 'IRBFT-20') is due to the shortening of each internode in its main stem, rather than a decrease in the number of nodes (Morishita et al. 2010; Kasajima et al. 2012b, 2013). In addition to decreased height, 'Darumadattan' expands its leaf area in the latter half of its growth stage, resulting in dry matter production and yields that do not differ significantly from those of standard Tartary buckwheat variety (Kasajima et al. 2012b, 2014). Furthermore, the rooting ability of 'Darumadattan' is superior to that of the standard-height variety (Kasajima et al. 2015). These reports indicate that the lodging resistance of 'Darumadattan' is extremely high and its cultivation is practical. For example, 'Darumadattan' did not lodge

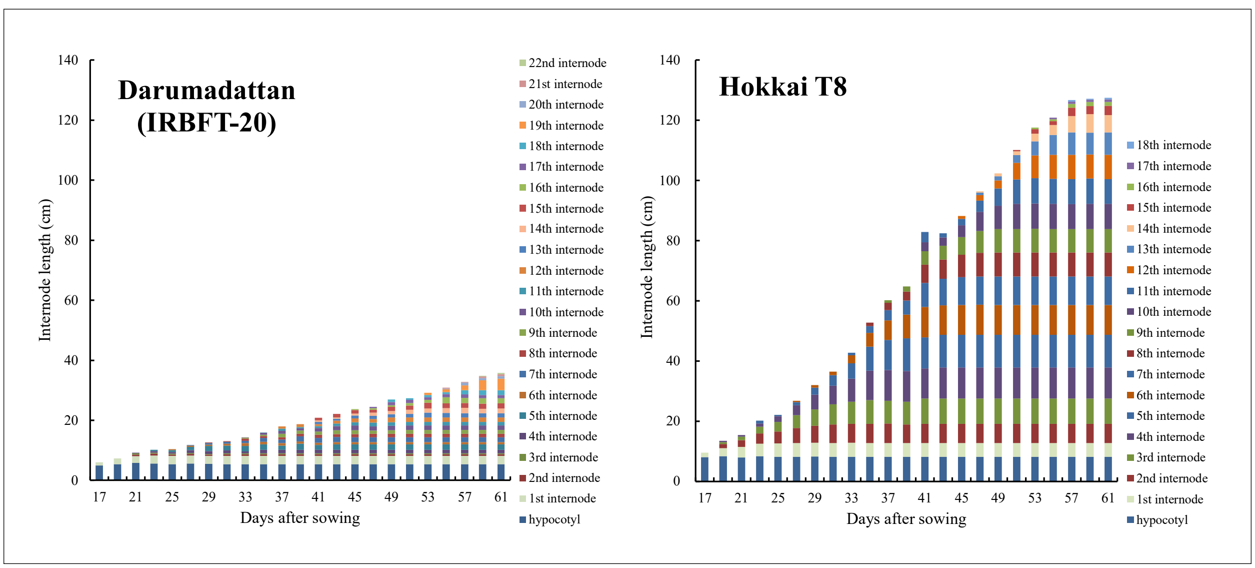

Fig. 2. *Changes in the internode lengths of the semidwarf Tartary buckwheat cv. 'Darumadattan' ('IRBFT-20') and its original cv. 'Hokkai T8' (Kasajima et al. 2013). The plants were grown in pots. 'Darumadattan' was known as 'IRBFT-20' during the breeding process.*

even after strong winds of a typhoon in Japan (Shimizu et al. 2020). In addition, the semidwarf trait in 'Darumadattan' is unique, and it can be used for future Tartary buckwheat breeding efforts. This trait will play an important role in the development of efficient cultivation techniques.

CONCLUSION

The present paper described some recent advances in knowledge regarding the nutritional, functional, and agronomic traits of Tartary buckwheat. In particular, the nonbitter and rutinosidase-deficient variety 'Manten-Kirari' and the semidwarf, lodging-resistant variety 'Darumadattan' are seen as innovative varieties that will have positive impacts on Tartary buckwheat-based industries. Although much is known regarding the nutritional function of Tartary buckwheat, knowledge on its yield performance and cultivation techniques is still limited. Further agronomic studies on Tartary buckwheat are necessary to increase its economic utilization. Knowledge generated from such studies will facilitate the creation of abundant and stable supplies of Tartary buckwheat products that will benefit the health of its consumers.

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IZVLEČEK

Novi dosežki v zvezi s prehranskimi, funkcijskimi in pridelovalnimi lastnostmi tatarske ajde (*Fagopyrum tataricum* (L.) Gaertn.)

Tatarska ajda (*Fagopyrum tataricum* (L.) Gaertn.) je pomemben vir za funkcijsko hrano, saj imajo zrna več polifenolov (na primer rutina) v primerjavi z navadno ajdo. Toda zaradi izrazite grenkosti in težav pri pridelovanju ima pridelovanje in predelava za prehranske izdelke le omejene možnosti. Prehransko in funkcijsko pomemembna snov tatarske ajde

je kvercetin, ki povzroča grenkost izdelkov. Kvercetin nastane kot posledica delovanja encima rutinozidaze (encim, ki razgrajuje rutin). V novejšem času smo razvili sorte tatarske ajde, ki so osnova za izdelke, ki niso grenki, saj imajo rutinozidazo le v sledovih. Kljub temu razvoju je še premalo informacij o možnostih pridelovanja tatarske ajde za izdelke brez grenkobe. Pri pridelovanju tatarske ajde je lahko pomemben problem poleganje rastlin, zato je bil razvit na poleganje odporen kultivar tatarske ajde s krajšimi internodiji in nižjo rastjo. V tej razpravi so povzeti novejši dosežki v zvezi s pridelovanjem in prehranskimi lastnostmi tatarske ajde.

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Research paper

Response of common buckwheat and Tartary buckwheat from different elevations to selenium treatment

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ABSTRACT

Common buckwheat and Tartary buckwheat were grown in Slovenia outdoors at different elevations – 300 m, 600 m and 1180 m a.s.l. Both species were foliarly treated with selenium twice (in the vegetative phase and in the flowering phase). The effects of Se treatment and different growing locations on selected biochemical, physiological and anatomical traits were monitored. In Se treated common buckwheat, amount of chlorophylls was higher in plants from Ljubljana (the lowest elevation – 300 m a.s.l.) than in plants grown in Podbeže (600 m a.s.l.), whereas in control group, plants grown in Ljubljana contained more chlorophylls than plants from Javorje (the highest elevation – 1180 m a.s.l.). In both buckwheat species, Se alone did not affect amount of chlorophylls in any of location. In Se treated common buckwheat plants, the amount of UV absorbing compounds was the highest in plants, grown at the highest elevation. In common buckwheat, Se lowered the number of CaOx in plants, grown in Javorje. Conditions at different elevations, as well as treatments with Se, did not affect potential and effective photochemical efficiency of Photosystem II.

Ključne besede: navadna ajda, tatarska ajda, nadmorska višina, selen, morfološke značilnosti, biokemijske značilnosti

IZVLEČEK

Navadno ajdo in tatarsko ajdo smo na prostem gojili na različnih nadmorskih višinah – na 300 m, 600 m in 1180 m n. m. Rastline obeh vrst smo dvakrat foliarno tretirali s selenom (v vegetativni fazi in v fazi cvetenja). Spremljali smo učinke tretiranja s Se in lokacije na izbrane biokemijske, fiziološke in anatomske lastnosti rastlin. Pri navadni ajdi,

tretirani s Se, iz Ljubljane (najnižja nadmorska višina – 300 m n. m.) je bila vsebnost klorofila večja kot v rastlinah iz Podbež (600 m n. m.), rastline iste vrste iz kontrolne skupine iz Ljubljane pa so vsebovale več klorofila kot tiste iz Javorja (najvišja nadmorska višina – 1180 m n. m.). Dodatek Se ni na nobeni lokaciji povzročil razlik v vsebnosti klorofilov v primerjavi s kontrolno skupino. Vsebnost UV absorbirajočih snovi v s Se tretirani navadni ajdi je bila največja v rastlinah, gojenih na najvišji nadmorski višini. Na omenjeni nadmorski višini je Se znižal število kristalov Ca-oksalata v navadni ajdi. Niti razmere na različnih nadmorskih višinah niti dodatek Se niso vplivali na potencialno in dejansko fotokemično učinkovitost fotosistema II.

INTRODUCTION

Buckwheat (*Fagopyrum*), a genus of dicotyledons from the family Polygonaceae, has spread to Europe and elsewhere in the world from southwest China. The area of origin of wild ancestors of the most nutritionally important species of buckwheat today - common buckwheat (*F. esculentum* Moench) and Tartary buckwheat (*F. tataricum* (L.) Gaertn.) - are supposedly the Chinese provinces of Yunnan and Sichuan (Ohnishi, 1998). It was recently reported that Tartary buckwheat originated in western China (Zhang et al., 2021). *F. esculentum* and *F. tataricum* are used in human nutrition, while the wild species *Fagopyrum cymosum* is used in traditional Chinese human and veterinary medicines (Luthar et al., 2021). Buckwheat is considered as pseudocereal (Huda et al., 2021) and belongs to the family Polygonaceae (Martinčič et al., 2007). Buckwheat has many health benefits for humans due to its contents of resistant starch, mineral elements, proteins, and especially phenolic substances, which prevent the effects of several chronic human diseases, including hypertension, obesity, cardiovascular diseases, and gallstone formation (Luthar et al., 2021). Flavonoids have a beneficial effect on human health (Panche et al., 2016). They play an important antioxidant role (Treml and Šmejkal, 2016). A nutritionally important flavonoid with strong antioxidant activity is rutin. It is present in many plants, with buckwheat standing out among plants in terms of its content (Kreft et al., 2002). Different parts of the buckwheat plant vary in rutin content: it is present in leaves and flowers in higher concentrations than in seeds, but the latter can nevertheless contribute significantly to the daily intake of flavonoids in the human diet (Kreft et al., 2002). Differences in rutin content are also present between different types of buckwheat.

Plants from Polygonaceae family contain numerous calcium oxalate (CaOx) druses (Nakata, 2012). The biological functions of CaOx druses in plants are not completely understood (Franceschi et al., 2015). They take part in the regulation of the bulk free Ca levels in plant tissues and organs, protection against herbivory and detoxification (e.g. heavy metals) (Franceschi and Nakata, 2005). Other possible functions of CaOx druses include role in regulation of ion balance, providing mechanical support in plant tissues and gathering and reflection of light (Nakata, 2012).

The climate of the Alpine region is very complex. The altitudinal gradient is related to decreased air temperature and pressure, increased precipitation, and changes in wind exposure, soil fertility and duration of snow cover (Caldwell et al., 1980). The increase in UV-B radiation (290-320 nm) ranges from between 6 and 8% (Caldwell et al., 1980) to 20% (Blumthaler et al., 1993) per 1,000 m of elevation.

Radiation with different wavelengths has different effects on plant growth and development. They can stimulate photosynthesis, activate specific photoreceptors and/or cause in most cases harmful photomodifications of macromolecules (Verdaguer et al., 2017). Both photosynthetic active radiation as well as UV-A and UV-B radiation affect Photosystem II (PSII). Their excessive intensities can lead to excessive excitation of PSII reaction centers and photooxidation damage to thylakoid membranes (Štroch et al., 2008). UV radiation triggers defense mechanisms in most plants that involve the synthesis of UV-absorbing substances, such as flavonoids (Štroch et al., 2008; Lim et al., 2021). Flavonoids are polyphenolic substances that perform various functions in plants: they protect plants against UV-B radiation, they are important for attracting plant pollinators, and play a role in allelopathy (Rozema et al., 1997). They also act as antioxidants and increase plant resistance to disease; some, for example, have antifungal effects (Harborne and Williams, 2000).

While selenium (Se) is known to be essential for animals, there is currently no evidence of its essentiality for plants (Hasanuzzaman et al., 2020; Trippe III and Pilon-Smits, 2021). However, it can be classified among beneficial elements. These are elements that can have a stimulating effect on plant growth in certain (low) concentrations, but are not necessarily necessary for plants (Pilon-Smits et al., 2009). Selenium plays important roles in human and animal organisms (Smoleń et al., 2014). Treating plants with Se can have a stimulating effect on their growth and development (Hasanuzzaman et al., 2020; Rady et al., 2020). It can increase their antioxidant capacity and resistance to biotic (Trippe III and Pilon-Smits, 2021) and abiotic stress such as drought (Rady et al., 2020). Selenium could be also added for the preparation, or supplemented to the diet (Germ et al., 2009).

Aim of the present paper was to find out the response of two species of buckwheat to conditions at different elevations and possible effect of adding selenium to their biochemical, physiological and anatomical characteristics.

MATERIALS AND METHODS

Experimental set up

Plants of common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*F. tataricum* (L.) Gaertn.) were grown from seeds in different locations with different elevations: in Ljubljana (300 m above sea level), in Podbeže, the municipality of Ilirska Bistrica (600 m above sea level) and in Javorje, the municipality of Črna na Koroškem (1180 m above sea level). Half of the experimental plants were foliarly treated twice with solution of potassium selenate (K2SeO4) with Se concentration of 10 mg L^{-1}. First treatment was at the vegetative phase (seedling age 40 days) and the second treatment was at the beginning of the flowering phase. Scheme of the experiments in Ljubljana, Podbeže and Javorje, is illustrated below (Fig. 1).

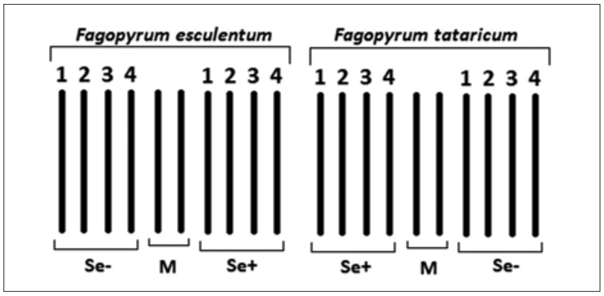

Fig. 1: *Schematic representation of the experimental field. An individual vertical represents a row of plants. The designation Se+ means treatment with selenium, Se- means the control group (without treatment with selenium), M means group of each species, where plants were not treated with Se, and samples were not taken.*

Biochemical and physiological analyses

The content of chlorophyll a, b and carotenoids was determined following the method by Lichtenthaler and Buschmann (2001a and 2001b). Weighed piece of fresh leaf was homogenized in a mortar and extracted in 6 mL acetone (90%). Homogenate of samples, extracted in 90% acetone was centrifuged for 4 minutes at 4000 rpm

Fig. 2: *Experimental site at Stopar family ecological farm "Mežner" in Javorje (1180 m above sea level), near Črna na Koroškem. At the left Tartary buckwheat plants, right common buckwheat plants.*

and 4 °C. After centrifugation, the volume of the extract was measured. Then extinction measurements using a VIS spectrophotometer (Lambda 12; PerkinElmer, Inc., Waltham, MA, USA;) at different wavelengths (470 nm, 645 nm and 662 nm) were done. Using the measured extinction values, the content of chlorophyll a and b and carotenoids per unit dry weight of the sample was calculated.

The anthocyanin levels were determined according to Lindoo and Caldwell (1978). The lyophilized plant tissue was first grounded. The samples were covered and incubated for 48 hours in a dark at a temperature of 3-5 °C. The absorbances of the extracts were measured at 530 nm using a UV/VIS spectrometer (Lambda 25; PerkinElmer, Inc., Waltham, MA, USA). The anthocyanins were extracted from the weighed dry plant material with HCl:methanol:water = 1:79:20 (v/v/v). The homogenate was centrifuged for 4 minutes at 4000 rpm and 4 ° C. The absorbance of the extracts was measured at 530 nm using a UV/VIS spectrometer (Lambda 25; PerkinElmer, Inc., Waltham, MA, USA). The anthocyanin levels are expressed in relative units. After centrifugation, the volume of the extract was measured.

For UV-absorbing substances, measurements were made at wavelengths from 280 to 400 nm. The sum of absorption values in the range 280-315 nm was used to calculate UV-B absorbing compounds, and the sum of absorption values in the range 316-400 nm was used to calculate UV-A absorbing compounds.

The photochemical efficiency of PSII, measured by Fv/Fm ratio (Fv/Fm = (Fm –Fo)/Fm) was measured using a portable chlorophyll fluorometer (PAM 2500; Walz, Effeltrich, Germany). Fv is a variable fluorescence, Fo and Fm are minimal and maximal chlorophyll a fluorescence yield in dark adapted sample. For dark adaptation, the samples were kept in cuvettes for 20 min before measurement. Fluorescence was excited with a saturating beam of "white light" (photosynthetic photon flux density, 8000 μmol m^{-2} s; 0.8 s). The effective quantum yield was determined by a saturating pulse of "white light" using a standard 60o angle clip, under saturating irradiance. Measurements were done at the prevailing ambient temperature. The yield coefficient was defined as follows: Y = (Fm' – F0')/Fm'; Fm' is the maximum and F0' the minimum fluorescence of an irradiated sample (Schreiber et al., 1998).

Analyses of CaOx druses

The numbers of the CaOx druses were determined on transverse sections using light microscopy (CX41; Olympus, Japan) with a digital camera (XC30; Olympus, Japan) and the CellSens software (Olympus, Japan).

RESULTS AND DISCUSSION

Conditions at different elevations have minor effects on the amount of chlorophylls, and UV absorbing compounds (Table 1).

Selenium was reported to enhance, lower or to not have any effect on the amount of chlorophylls. Photosynthetic pigments of cowpea leaves were significantly enhanced by the foliar application of Na selenate up to 25 μM, but inhibited at high concentrations (50 μM) (El Lateef Gharib et al., 2019). In another study (Golob et al., 2018b), Se in the same concentration as in the present study increased the chlorophyll a and b and carotenoid contents per mm^2 of leaf area in Tartary buckwheat. On the other hand, it was recently reported that foliar spray-

Table 1: *Biochemical, physiological and anatomical characteristics in Se-treated and control common buckwheat leaves.*

		Common buckwheat – treatment:					
		Se+			Se-		
Parameter	**Unit**	**Ljubljana**	**Podbeže**	**Javorje**	**Ljubljana**	**Podbeže**	**Javorje**
Total chlorophylls	mg/g dm	18,8 ± 1a	12,7 ± 0,9c	16,2 ± 1,9a,b,c	18,1 ± 1,7a,b	14,3 ± 1,1b,c	13,6 ± 0,3c
UV-absorbing compounds	relative units	255± 21b	218 ± 19b	360 ± 48a	242 ± 33b	226 ± 18,b	275 ± 15b
No. of CaOx druses	/	23,6 ± 0,7a	18,4 ± 1,6b,c	18 ± 1,5c	22,6 ± 0,3a	20,6 ± 0,7a,b,c	21,5 ± 0,7ab

Data are means ± standard error (n = 4 for each treatment). Different letters indicate significant differences between different treatments, (p < 0,05; Duncan Post Hoc Test).

Legend: *Se+, added selenium; Se-, no added selenium; dm, dry matter* ing with 10 mg L^{-1} Se in the form of selenite and selenate, did not have any effects on the amount of chlorophyll a and chlorophyll b in chicory (Germ et al., 2020). The latter was evidenced also from the present study, where plants were foliarly sprayed with the same concentration of Se (Table 1, Table 2). However, there were some differences in amount of chlorophylls between plants of the same species and treatment from different elevations (Table 1, Table 2). In Tartary buckwheat, the only difference regarding the amount of chlorophylls was present between untreated plants from Javorje and Podbeže, with latter having lower amount of chlorophylls (Table 2). However, this was not the case in common buckwheat. In untreated common buckwheat plants from Javorje, amount of chlorophylls was lower than in untreated plants grown in Ljubljana. In Se treated plants of the same species from Ljubljana, amount of chlorophylls was higher than in plants of the same treatment from Podbeže (Table 1). It seems that in certain cases plants from higher elevations contained lower amount of chlorophylls. Similar results were given by Roblek et al. (2008), who studied biochemical reponse of *Hypericum perforatum* to the conditions at different elevations. In Se treated common buckwheat plants, the amount of UV-absorbing compounds was the highest in plants, grown at the highest elevation. UV-absorbing compounds protect plants from potential damage (Van de Staaij et al., 1995). The highest amounts of UV absorbing compounds were measured also in in *Hypericum perforatum* from higher elevations (Roblek et al., 2008). In Tartary and common buckwheat plants from Javorje, Se treatment induced the accumulation of UV-absorbing compounds (Table 1, Table 2). It was already known that Se increases contents of UV-absorbing compounds and anthocynins in hybrid buckwheat (Golob et al., 2018a). In common buckwheat, Se lowers the number of CaOx in plants, grown in Javorje (Table 1). Similar results were given by Golob et al. (2018b) for Tartary buckwheat, where Se decreased the density of CaOx druses in plant leaves. Toxic metals (cadmium, lead, copper and zinc) had an effect on mechanisms that mediate crystal formation in *Corchorus olitorius* (jute) and *Malva parviflora* (cheeseweed) (Faheed et al., 2013). Maybe similar case for the negative effects of Se on CaOx druses synthesise was present also in common buckwheat in the present study. In Se treated common buckwheat plants, the number of CaOx druses was highest at the lowest elevation (Table 1), whereas in Tartary buckwheat plants, the number of CaOx druses was higher in plants from Ljubljana than in plants from Podbeže (in Se treated plants), and in plants from Ljubljana than in plants from Javorje (in untreated plants) (Table 2).

Values of potential photochemical efficiency of PSII ranged between 0,572 and 0,807 (average value 0,752) in common buckwheat, and between 0,647 and 0,820 (average value 0,765) in Tartary buckwheat (data not shown). This is indicator that all experimental plants had good fitness, and also that neither conditions at different elevations nor the addition of Se posed stress to the common and Tartary buckwheat. It has already been shown in many studies that Se does not influence the potential photochemical efficiency of PSII, such as in cihory, treated with 10 mg Se L^{-1} (Golob et al., 2020), and red cabbage plants treated with 2 µg L^{-1} Se (Mechora et al., 2011).

To conclude, we presume that both species are adapted to the conditions present at higher elevation. In addition, neither Se in concentrations used in this study nor different elevation posed stress to the plants. Se treatment increased the amount of UV-absorbing compounds

Table 2: *Biochemical, physiological and anatomical characteristics in Se-treated and control Tartary buckwheat leaves.*

		Tartary buckwheat – treatment:					
		Se+			Se-		
Parameter	**Unit**	**Ljubljana**	**Podbeže**	**Javorje**	**Ljubljana**	**Podbeže**	**Javorje**
Total chlorophylls	mg/g dm	17,6 ± 3a	16 ± 1,2a,b	17,7 ± 1,6a	13,3 ± 2a,b	10,6 ± 1b	17,3 ± 1,7a
UV-absorbing compounds	relative units	258 ± 17a,b	225 ± 55a,b	293 ± 19a	206 ± 29a,b	216 ± 36a,b	166 ± 27b
No. of CaOx druses	/	20 ± 2,2a	13,4 ± 0,6b	16,3 ± 2,3a,b	20,7 ± 1,4a	16,9 ± 1,8a,b	13,8 ± 1,6b

Data are means ± standard error (n = 4 for each treatment). Different letters indicate significant differences between different treatments, (p < 0,05; Duncan Post Hoc Test).

Legend: *Se+, added selenium; Se-, no added selenium; dm, dry matter* in plants of both species grown at the highest elevation, and decreased the number of CaOx druses in common buckwheat from the highest elevation. However, Se did not affect the amount of chlorophylls in plants of neither species. There were some differences in the amount of chlorophylls between common buckwheat plants as well as between Tartary buckwheat plants from different elevations. The number of CaOx druses was highest in Se treated common buckwheat plants from the lowest elevation. In untreated Tartary buckwheat plants from the lowest elevation, it was higher than in plants from the highest elevation. In Se treated Tartary buckwheat grown at the lowest elevation, the number of CaOx druses was higher than in plants of the same species and treatment from Podbeže. Based on the results of photochemical efficiency of PSII measurements, experimental plants of both buckwheat species from Se treated and control group from all locations were in good physiological condition.

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IZVLEČEK

Odgovor navadne in tatarske ajde na različnih nadmorskih višinah na dodatek selena

Namen raziskave je bil preučiti odziv dveh vrst ajde na razmere, ki so na različnih nadmorskih višinah, ter morebiten vpliv foliarnega dodatka selena (Se) na njihove biokemijske, fiziološke in anatomske lastnosti. Navadno ajdo (*Fagopyrum esculentum* Moench) in tatarsko ajdo (*Fagopyrum tataricum* (L.) Gaertn.) smo posejali na prostem na treh lokacijah z različnimi nadmorskimi višinami (300 m, 600 m in 1180 m. n. m.) ter ju foliarno tretirali s kalijevim selenatom (10 mg Se L-1). Spremljali smo izbrane biokemijske, fiziološke in anatomske lastnosti ter primerjali rezultate med tretirano in kontrolno skupino ter med rastlinami, vzgojenimi na različnih nadmorskih višinah. Statistične analize smo izvedli znotraj posamezne vrste. Dodatek Se je povečal vsebnost UV absorbirajočih snovi pri obeh vrstah ajde, gojenih na najvišji nadmorski višini. Tretirana navadna ajda s te nadmorske višine ima najvišjo vsebnost UV absorbirajočih snovi. Se je zmanjšal število kristalov Ca-oksalata pri navadni ajdi, ki je uspevala na najvišji nadmorski višini. Dodatek Se ni vplival na vsebnost klorofila. Med rastlinami iste vrste in tretmaja so bile prisotne sledeče razlike v vsebnosti klorofilov: tretirane rastline navadne ajde iz Ljubljane (najnižja nadmorska višina) so vsebovale več klorofilov kot tiste iz Podbež, netretirane rastline iz Ljubljane pa več klorofilov kot tiste iz Javorja (najvišja nadmorska višina). Netretirane rastline tatarske ajde iz Javorja so vsebovale več klorofila kot rastline iz Podbež. Pri obeh vrstah ajde smo zabeležili nekaj razlik v številu kristalov Ca-oksalata med rastlinami iste obravnave na različnih nadmorskih višinah. Pri tretirani navadni ajdi je bilo njihovo število največje na najnižji nadmorski višini. Tam je bilo število kristalov Ca-oksalata tudi pri netretirani tatarski ajdi večje kot v Javorju. Pri tretirani tatarski ajdi je bilo število kristalov Ca-oksalata pri rastlinah iz Podbežah nižje kot pri rastlinah iz Ljubljane. Glede na rezultate meritev fotokemične učinkovitosti fotosistema II, ki so pokazale dobro fiziološko stanje rastlin obeh vrst iz tretirane in kontrolne skupine na vseh treh nadmorskih višinah, lahko zaključimo, da so poskusne rastline dobro prilagojene na razmere na različnih nadmorskih višinah in da dodatek Se za rastline ni predstavljal stresa.

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