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Content of total proteins, starch and proline in seeds of some wild relatives of cultivated plants

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Abstract

Revealing of interesting features among wild relatives of cultivated plants is prerequisite for their direct cultivation or application as a genetic material. Wild legumes and cereals of Georgia are not studied from this point of view. Accordingly, the purpose of the presented investigation was to study some, interesting from agricultural point of view, biochemical indices – content of total proteins, starch and proline, as well as total antioxidants activity - in seeds of some wild cereals and legumes. Plants species: *Hordeum leporinum* Link – mouse barley, *Aegilops cylindrica* Host - jointed goat grass, *Avena fatua* L. - common wild oat, *Anisantha sterilis* (L.) Nvsky- sterile brome, *Poa palustris* L. - fowl meadow grass, *Agropyron repens* (L.) P. Beauv.- quackgrass, and *Pisum arvense* (L.) Asch. – field pea served as test objects. *Triticum aestivum* L. – common wheat and *Pisum sativum* L. - pea were selected among the cultivated species for the comparative analysis of studied indices. Tested wild herbs may be grouped as interesting objects from agricultural and medicinal points of view, according to experimental results. Field pea and jointed goatgrass (seeds are rich of both starch and proteins), mouse barley and wild oat (seeds are rich of starch), as well as sterile brome (seeds contain enough amount of proteins) may be used in breeding and agricultural purposes, because of high content of proteins and starch in seeds. High content of proline in seeds and total antioxidant activity make wild oat, sterile brome, fowl meadowgrass and quack grass as interesting objects from medical point of view.

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Introduction

Agricultural products industry relies on the total genetic resources of the world plants, which is formed by the wild relatives, together with cultivated crops. Studying of the wild relatives of cultivated plants still becomes popular inspite of a multi southand years history of plants domestication by the man (Fernandez-Marin *et al.*, 2014; Cooper, 2015; Zhu, 2017). This fact is stipulated by the problem of world's population feeding, and by the progressing manifestations of climate global change (Ford-Lloyd *et al.,* 2011). Investigation of wild relatives aims revealing of new plant species with valuable nutritional properties, on the one hand, and struggle for retention of world's biodiversity, which is endangered by climate change and man's activity, on the other (Ford-Lloyd *et al.,* 2011).

Domestication of plants diminished their genetic diversity and became the reason of their vulnerability to environment. Thus, climate global change will result in new problems of agricultural crops growing, as they will appear less resistant to increased environmental stresses, like drought, very high or low temperatures etc. (Rowlands *et al.,* 2012; Meyer *et al.,* 2012).

Cultivation of plants became the reason for decline or disappearance a number of useful features, as well, because the breeder paid less attention to nutritional properties of seed and preferred other features like taste, productivity, resistance to storing, etc. (Fernandez-Marin *et al.,* 2014; Warschefsky *et al.,* 2014); e.g. comparable studies of carotenoides and fatty acids in some legumes revealed decrease of these substances in domesticated ones (Fernandez-Marin *et al.,* 2014).

Thus, revealing of interesting features of nutritional value among wild relatives of cultivated plants is prerequisite for their direct cultivation or further application as a genetic material. Wild legumes and cereals of Georgia are not studied from this point of view. Accordingly, the purpose of our investigation was to study some interesting from agricultural point of view biochemical indices – content of total proteins, starch and proline, as well as total antioxidant activity in seeds of some wild cereals and legumes, growing in Georgia. These investigations will make possible to reveal the material for breeding and genetic experiments among the wild species of Georgia.

Materials and methods

Plant material

Plants species: *Hordeum leporinum* Link – mouse barley, *Aegilops cylindrica* Host - jointed goatgrass, *Avena fatua* L. - common wild oat, *Anisantha sterilis* (L.) Nvsky- sterile brome, *Poa palustris* L. - fowl meadowgrass, *Agropyron repens* (L.) P. Beauv.- quackgrass, *Pisum arvense* (L.) Asch. – field pea served as test objects. *Triticum aestivum* L. – common wheat and *Pisum sativum* L. - pea were selected among the cultivated species for the comparative analysis of studied indices. Wild plants were picked in different habitats: environs of the National Botanical Garden in Tbilisi, surroundings of salt lakes of Gareji desert (Sagarejo region, Georgia), Varketili (suburb of Tbilisi). The cultivated plants were collected on the experimental plot, situated on the territory of National Botanical Garden in Tbilisi.

Hordeum leporinum Link (mouse barley) – is annual weed grass. It is a good fodder for cattle in regions with water deficiency. Though, because of rough awns the ripen seeds are dangerous for animals – it may cause irritation of digestive system. Seeds may be boiled or milled and used for preparing porridges and bread. Herb decoction is used to cure urinary bladder (Tanaka, 1976; Moerman, 1998; Johnston *et al.*, 2009).

Aegilops cylindrica Host (jointed goatgrass) – is annual grass, resistant to unfavorable conditions: drought, low temperature, salinization. It grows on poor, stony, or polluted places, near roads. In mountains jointed goatgrass may reach 2000m height above sea level. It is regarded as good fodder for livestock. The plant is used in wheat breeding, to receive new sorts (Donald and Ogg, 1991).

Avena fatua L. (common wild oat) is a cosmopolitian plant, grows everywhere, especially in cereal fields.

According to one of the considerations, *A. fatua* is the ancestor of a cultivated oat (Scholz, 1991). It had been applied for the formation of disease resistance in wheat (Tang *et al.*, 1997); e.g. the tetragenic hybrids of wheat with wild oat are stable against leaf rust (*Puccinia recondita*), powdery mildew (*Erysiphe graminis*), root rot (*Alternaria alternata*) and other deseases.

Common wild oat is used both in folk and traditional medicine. From unripe seeds the ethanol tincture is prepared as tonic, laxative and nervous system stimulant, as well as antidepressant, against the epilepsy, nervous exhaustion, and drug addiction. It has diuretic, and temperature lowering effect (Moore, 1989). Seeds of common wild oat are used as food as well. They are milled into gentle, yellowish flour, which is used in bakery, and preparing sweets. Seeds may be used in germinated state, boiled or raw. The substitute of coffee is prepared from roasted seeds (Moore, 1989).

Anysantha sterylis (L.) Nevsky (sterile brome) is annual grass. grows on waste places, near roads. The plant is regarded as a harmful agricultural weed in Mediterranean region. (Cooper and Moerkerk, 2000; Hamal *et al.*, 2001). Sterile brome mainly grows in plantations of cereals, sugar beet, sunflower, soybean, lens. It is resistant to drough and wind; feels well on all type of soils (INRA, 2001). *Poa palustris* L. (fowl meadowgrass) – is a perennial grass, good as hay, resistant to soil salinization (Gubanov *et al.*, 1990).

Agropyron repens (L.) P. Beauv. (quackgrass) – is a perennial grass, annoying weed of vegetable gardens and orchards, is used as livestock fodder. Its rhizomes are used in medicine as diuretic, diaphoretic and expectorant, antiinflammatory and slight laxative (Vogl *et al.*, 2013; Hubbard, 1978). *Pisum arvense* (L.) Asch. (field pea) – annual herb, domesticated for about 7000 years. It is used as human and animal food; is cold resistant (McKay, Schatz, 2003).

Starch

Starch content in seeds was determined colorimetrically (Pochinok, 1976). Seed powder was mixed with 80% solution of Ca $(NO_3)_2$, boiled for 3-5min and centrifuged. Part of the supernatant was added 0.5% solution of iodine. Obtained sediment was centrifuged, washed with 5% solution of Ca $(NO_3)_2$. Washed sediment was added 10ml of 0.1N NaOH and was hold on a boiling water bath for 5min. Obtained solution was added 0.3 ml of 0.5% iodine and 2 ml of 1M HCl. The optical density of the blue solution was measured at 580-560nm.

Total protein assay

Content of proteins was determined after Lowry (1951). *Proline* 0.5g of dry leaves were mashed in 10ml of 3% sulphosalicylic acid and filtered. 2ml of the filtrate was added to 2ml of acid ninhydrin and 2ml of ice acetic acid. After 1h exposition on a water bath the extract was cooled and added with 4ml of toluene and divided in a separating unnel. Optical density of upper layer was measured on a spectrophotometer (SPEKOL 11, KARL ZEISS, Germany) at 520 nm (Bates *et al.*, 1973).

The total antioxidant activity

This index was measured by modified method using diphenyl-picryl-hydrazyl (DPPH) (KolevaII *et al.,* 2002). 200mg of experimental powder was extracted two times with 96° ethanol. Obtained extract was evaporated on a water bath and the remained sediment was dissolved in 10ml of water- alcohol mixture. 0.01ml of the obtained solution was added with 4ml of 40μM DPPH solution and after 30 minutes of incubation in the dark the optical density was measured at 515nm. The percent of inhibition was calculated.

Statistical analysis

One way ANOVA and Tukey's multiple comparison tests were used to test differences between the means. All calculations were performed using statistical software Sigma Plot 12.5.

Results and discussion

Starch

Starch is one of the significant carbohydrates both in human and animal diet. It has complex industrial application as well (Burrell, 2003).

Thus, high content of starch in seeds significantly raises its nutritional value. Among the tested plants the highest content of starch was discovered in jointed goatgrass (Fig.1). No statistical difference was found between the results of common wild oat and wheat (p=0.16), pea and common wild oat (p=0.53), and field pea and mouse barley (p=0.5) (Fig.1). Statistically different results were revealed between the low starch containing species: sterile brome, fowl meadowgrass and quackgrass ($p<0.05$). According to experimental results high content of starch in seeds of jointed goatgrass, wild oat, field pea and mouse barley turns these weeds into interesting objects for agriculture in future.

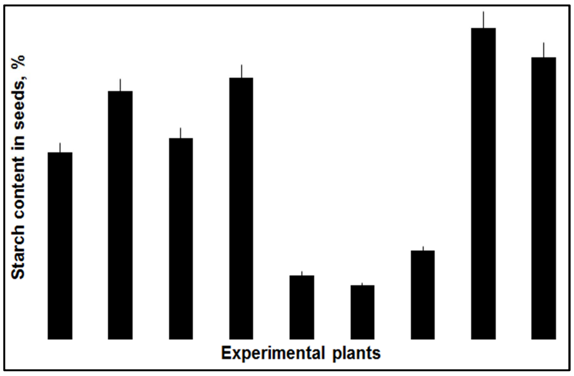

Fig. 1. Starch content in experimental plant seeds.
1. Field pea 2. Pea 3. Mouse barley 4. Wild oat 5. Sterile brome 6. Fowl meadowgrass 7. Quackgrass 8. Jointed goatgrass 9. Wheat.

Total proteins

Besides the high nutritional value, proteins play a role of food antioxidant additives because of their inhibitory effect on lipid-oxidation. The bioactive peptides like soy bean, casein, gelatin, serum protein and wheat gluten, received as a result of hydrolysis of different food-proteins, possess antioxidant properties (Elias *et al.*, 2008). The highest content of total proteins among the studied species was revealed in seeds of pea, field pea and jointed goatgrass. The lowest index had mouse barley, wild oat, quackgrass and fowl meadowgrass (the results did not differ statistically, $p>0.05$) (Fig. 2). The index was twice lower in wheat and three fold lower in sterile brome compared to the maximal result. By the content of proteins in seeds among the studied species may be distinguished pea, jointed goatgrass and sterile brome which may be used in future as material for hybridization and genetic experiments.

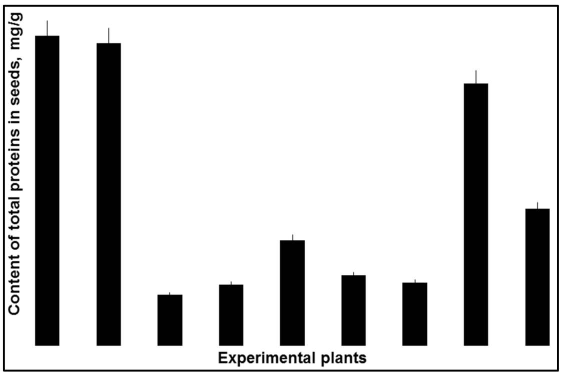

Fig. 2. Content of total proteins in seeds of experimental plants.
1. Field pea 2. Pea 3. Mouse barley 4. Wild oat 5. Sterile brome 6. Fowl meadowgrass 7. Quackgrass 8. Jointed goatgrass 9. Wheat.

Proline

Proline has multiple functions in plant stress adaptation, protection and signal transduction. It plays a role of metabolic signal, which regulates metabolites pool, expression of a number of genes and affects totlal plant growth and development (Szabados and Savoure, 2009).The positive correlation between proline accumulation and seedling vigor as well as plant resistance has been demonstrated (Borzouei *et al.,* 2012). Thus, one can judge about plant stress resistance by the proline content in seeds.

Proline is useful and important substance for animals, which receive it together with food. It is necessary for collagen and cartilage formation and keeps muscles and joints flexible. Thus, proline additives may be useful in cases of osteoarthritis, persistent soft tissue strains, and chronic back pain (www.vitaminstuff.com/amino-acid-proline.html).

The highest content of proline was established in seeds of sterile brome and fowl meadowgrass. The lowest content of substance was discovered in seeds of jointed goatgrass, wheat and wild oat. No statistical differences were found between field pea, mouse barley and quackgrass on the one hand ($p>0.05$), and wheat, jointed goatgrass and wild oat, on the other (p>0.05). High content of prolin in seeds makes fowl meadowgrass, sterile brome, field pea, mouse barley and quackgrass as interesting objects for agriculture and medicine.

Total antioxidant activity

Antioxidants is a group of different class substances, which are responsible for detoxications and protection of biological structures (Sharma *et. al.,* 2012). Total antioxidant activity is a significant integrated index, which demonstrates the stress resistance of plant. Besides it may be used for evaluation of health-beneficial total antioxidant potential of plant.

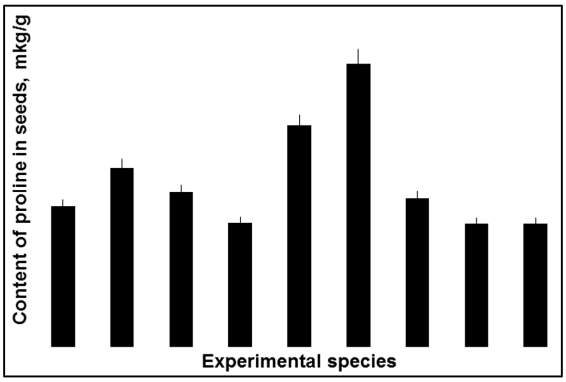

Fig. 3. Content of proline in experimental plant seeds.

1. Field pea 2. Pea 3. Mouse barley 4. Wild oat 5. Sterile brome 6. Fowl meadowgrass 7. Quackgrass 8. Jointed goatgrass 9. Wheat.

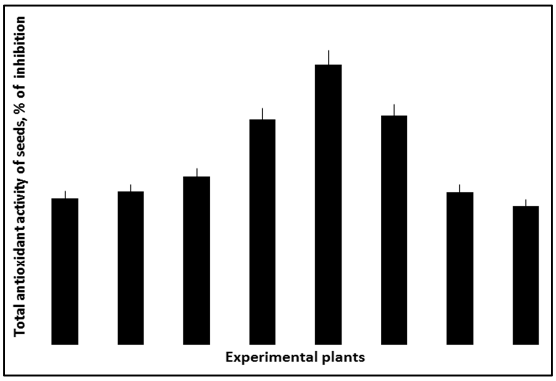

Fig. 4. Total antioxidant activity of experimental plant seeds.

1. Field pea 2. Pea 3. Mouse barley 4. Wild oat 5. Sterile brome 6. Fowl meadowgrass 7. Quackgrass 8. Jointed goatgrass

The highest index of total antioxidant activity was revealed in seeds of sterile brome, wild oat and fowl meadowgrass. Comparatively low and similar results were discovered in field pea, pea, quackgrass and jointed goatgrass seeds (p>0.05). In case of distinguished plants future application for agricultural or medical purposes, the high index of total antioxidant activity will essentially rise their economic value.

Conclusions

According to experimental results some species of tested plants may be distinguished by the studied indices. Seeds of field pea and jointed goatgrass were rich of both, starch and proteins. Seeds of mouse barley and wild oat were rich of starch; while seeds of sterile brome contained enough amounts of proteins. Accordingly, these plants may be recommended to be used for agricultural purposes as well as material for breeding experiments in future. High content of proline and total antioxidant activity of seeds as indicators of stress resistance of plant make wild oat, sterile brome, fowl meadowgrass and quackgrass as interesting objects for both agriculture and medicine.

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