Acta Agroph., 2016, 23(4), 569-582

SELECTED BREEDING CHARACTERS AND SEED PROTEIN CONTENT OF ADZUKI BEAN (*PHASEOLUS ANGULARIS* W.H. WHITE) GROWN IN CENTRAL EUROPE

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A b s t r a c t. In the years 2011-2012, thirteen cultivars and breeding lines of Chinese origin adzuki bean (*Phaseolus angularis* W.H. White) were evaluated for their selected breeding characters and seed protein content in the climatic conditions of Central Europe. In the field experiment, the evaluation included seedlings emergence, number of pods per plant, number of seeds per pod and plant health status. In the laboratory research, the assessments included seed germination as well as seed dry matter and total protein contents. In Western Poland climatic conditions, it was possible to produce seeds of adzuki bean. However, in cold and wet years, they did not set seeds. The tested materials differed in plant emergence, earliness, number of pods per plant, number of seeds per pod and plant health status. The produced seeds had germination capacity from 0 to 54% and no symptoms of bean weevil (*Acanthoscelides obtectus* Say) damage. They also had from 20.01 to 31.91% of total proteins in d. m., which proved they could be a valuable source of plant protein. Amongst the evaluated materials, one breeding line (marked with the letter J) turned out to be the best, whereas the other one (marked Z1) was also interesting for further breeding work.

K e y w o r d s: adzuki bean, *Vigna angularis,* bean breeding characters, seed germination, seed protein

INTRODUCTION

Beans have been known to man for thousands of years. Thanks to their high nutritional value, they have been a valuable source of protein. The genus *Phaseolus* includes over 230 species, divided into two main groups. The first one comprises

beans of American origin, with large seeds and valuable breeding properties. It is more numerous than the second one, and its cultivation is more widespread. The most important in this group are: common bean (*Phaseolus vulgaris* L.), runner bean (*P. coccineus* L.) and lima bean (*P. lunatus* L.). The latter group includes Asian beans with finer seeds and smaller market spread than the crops from the first group. The most important beans in this group are: mung bean (*P. aureus* Roxb.), rice bean (*P. calcaratus* Roxb.) and adzuki (or adzuki) beans (*P. angularis* W.H.Wight.) (Nowiński 1970). The oldest evidence of the beans cultivation in Asia dates back 6-8 thousand years. The first written reports concerning adzuki beans are dated 1,300 years ago. The Old World plants have been popular since the first Columbus expedition. The traveller brought to Europe the American beans which then gradually replaced those of the Asian origin (Korohoda 1969). The adzuki bean comes from the Far East, most probably from Manchuria in China (Gepts 1998, Isemura *et al*. 2001, 2001, Kaga *et al*. 2008, Xu *et al*. 2008). It is a well-known vegetable (Black *et al.* 2006) and a medicinal plant (Zhao *et al.* 2009, Yu *et al*. 2011, Zhang and Xue 2012). Its seeds have up to 29.2% of proteins (Lumpkin *et al*. 1993, Sun *et al*. 2006). The extracts of the seeds are used in the treatment of numerous inflammatory conditions in humans, especially of the kidney and bladder. It is also recommended in the diet of patients with heart disease and hypertension. They are also a component of the diet of athletes who run long distances (Yu *et al.* 2011). In clinical studies with rats, water extracts from the seeds of adzuki decreased blood cholesterol. Moreover, adzuki bean seeds contain tannins which act as antioxidants (Amarowicz *et al.* 2008). They have been used to cure many human malfunctions and diseases (Zhao *et al.* 2009, Zhang and Xue 2012).

Adzuki bean is an annual, self-pollinated and warm season crop (Sun *et al*. 2006). In good conditions, adzuki bean develops seeds with germination capacity over 85% and keeps it up to 4 years (Lumpkin *et al.* 1993, Wang *et al.* 1997, Sun *et al.* 2006). Its cultivation, use and main breeding characters have been presented in numerous papers (Liu and Zhou 2007, Zang *et al.* 2012, Liu 2011, Yin *et al*. 2011, Wu *et al.* 2011, Shen and Li 2012a).

The main directions of adzuki beans breeding are: high yield, resistance to drought, high amounts of proteins and low amounts of fats (Shen and Li 2012b, Wang *et al.* 2013, Gao *et al.* 2013).

China is the largest producer of adzuki bean in the world (Wang *et al.* 2013). The most common use of the species as food is to produce sweetened paste (Yousif *et al.* 2003). Adzuki bean was first grown in Poland in the years 2007-2008, but problems have occurred with testing mid-late and late cultivars which did not set seeds (Hołubowicz *et al.* 2009).

The main research goal of this study was to determine which of the 13 adzuki beans cultivars and breeding lines is the most promising and could be grown in the Polish climate, typical for Central Europe. Another important goal was to evaluate its basic breeding characters when grown in different climatic conditions.

MATERIALS AND METHODS

The seeds. The subject of the study were adzuki bean seeds (*Phaseolus angularis* W.H. White). The seeds of cultivars and breeding lines used in the experiments came from the Institute of Vegetable Crops, Chinese Academy of Agricultural and Forestry Sciences in Beijing, Institute of Vegetable of the Beijing town, Beijing Agricultural University, College of Agriculture of the City of Beijing, Dongbei Agricultural University in Harbin (the Heilongjiang province) and seed companies from the province of Heilongjiang, Shandong, Liaoning and Jilin. The material for the study was collected in the years 2006-2010.

Field experiment. The experiment was carried out at the Marcelin Experiment Station of the Faculty of Horticulture and Landscape Architecture, Poznań University of Life Sciences. The Station is located in Poznań, Western Poland, Central Europe. The soil used was podzolic with strong sand on clay. Before sowing the seeds, the field to be used in the experiment, i.e. used for agricultural purposes, was set aside. Just before sowing the seeds, the rotary tiller was used to destroy weeds. The size of the experiment plot was 15 m x 25 m.

The experiment was started twice: in May 2011 and in May 2012. After smoothing the soil, eighty rows were marked: 40 in each replication. Each row was 3.5 m long and the spacing between rows was 0.5 m. The seeds were dressed with chemical pesticide T 75 DS / WS and then sown. There were 40 seeds sown in each row and spacing in the row between individual seeds was 8 cm. Each cultivar and breeding line used in the experiment consisted of 40 seeds and was sown in 2 replications. The distance between replications on the field was 2 m. The field with the plants was not irrigated during the whole growing season. However, it was controlled chemically against the bean weevil (*Acanthoscelides obtectus* Say): with 10 ml of the insecticide Karate Zeon 050 CS and with 12 ml of the insecticide Falcon 460 EC used in 10 litres of water.

Breeding observations

During the vegetation of the plants in the field, their harvest, and processing, selected breeding characters were observed. These were: number of plants in a row, flower colour and number of pods per plant based on the data from five randomly selected plants from each row. Another important plants' feature evaluated was

their health status. In this case, the five class scale was used. The first class plants in a row were very contaminated with diseases or dried. The second class plants had high percentages of their leaves with symptoms of diseases. The following third class had medium healthy plants with only a few plants infested. The next fourth class had almost all healthy plants, with only individual leaves and pods with disease symptoms. The last fifth class had all healthy plants with no disease symptoms.

On October 15, 2011, and 2012, pods were collected from the field. Then, they were evaluated for the number of seeds per pod. To do so, 10 pods from each row were randomly chosen, opened, and the seeds in them were counted. The cultivars and lines whose seeds did not emerge and, thereby, produced no seeds, were not included in further research.

Weather conditions

Weather conditions in the field in Western Poland (Central Europe) in 2011 should be considered as favourable for the cultivation of beans. During conducting the experiments, three crucial plants vegetation periods were taken into account: the first one – from seed sowing to plant emergence and development of seedlings, i.e. May-June, the second one – flowering, i.e. July-August and the third one – setting and maturation of seeds, i.e. September-October. In the first period, the mean daily air temperature was higher than the average value of the previous six years. The sum of rainfalls in May 2011 was higher than the average sum for the years 2005-2010. In June, the difference was even bigger: 16 mm over the average sum. In the second period, the average daily air temperature in July was 16.7°C, and it was lower than the average value for the last 6 years. The monthly sum of rainfalls during this period was more than twice higher than the average sum of the previous six years. The average daily air temperature in August was higher than the average value recorded in the last 6 years. The average daily air temperature in September was only 0.3°C higher than the average value for the years 2005-2010, while in October – lower by 0.1°C. In turn, rainfall sum in September was 17.4 mm, and it was almost twice lower than the average value over the previous six years. In 2011, the sum of rainfalls in October was 20.6 mm. When compared to the average value of the years 2005-2010, it was lower by 5.4 mm. In 2012, the average daily air temperature for May and June was higher than the average over the previous six years. Total rainfall in May 2012 was 47.6 mm, compared to 60.1 mm average rainfall in May in the years 2005-2010. The total rainfall in June 2012 was 94.4 mm, and more than twice the average amount of rainfall for that month in 2005-2010. Air temperature in July was lower than the average temperature for this month, and in August – slightly higher. Rainfalls in July almost doubled the average of this weather character in the

years 2011-2012. The average air temperature in September 2012 was slightly lower than the calculated average value based on the data from the years 2005-2010.

Table 1. Average daily air temperatures and sum of rainfalls for each month, May-October, in the years 2011-2012, and the average values for 2005-2010

Period	Average daily air temperature (°C)			Rainfalls (mm)		
Month / year	2011	2012	2005-2010	2011	2012	2005-2010
May	14.6	15.6	13.8	9.4	47.6	60.1
June	18.7	16.2	14.7	55.0	94.4	38.6
July	16.7	19.3	20.6	146.4	126.2	64.5
August	18.8	18.7	18.4	30.0	33.4	62.2
September	15.1	14.4	14.8	17.4	20.6	33.3
October	8.8	10.0	8.9	20.6	18.0	26.0

Source: data from the Experimental Station of the Faculty of Horticulture and Landscape Architecture, Poznań University of Life Sciences, Western Poland, Central Europe

As a general statement, one can say that the weather conditions in 2011 were much better for growing beans than in 2012. In the latter year, the weather was favourable for other bean crops: common and broad beans, however, they were fatal for adzuki beans – the seeds in the field were not set at all or, if set, they did not have enough time to mature and eventually did not germinate.

LABORATORY TESTS

Germination evaluation

The germination test was carried out in 2012. At first, the seeds were disinfected with 10% ethanol. Then, 4 replications of 50 seeds for each cultivar and breeding line were prepared. Next, the seeds were placed between wet blotting paper rolled into rolls and incubated at 20°C. After 5 days of the incubation, all the seeds with sprouts longer than 5 mm were removed and the rolls were rolled back as before. After another 4 days, the evaluation was repeated. There were 4 groups of germs: normal, abnormal, healthy but not germinated (fresh seeds), and hard seeds.

Chemical analysis of the obtained seeds

For dry matter analyses of the seeds, at first, the seed sample was crushed to get a powder. Then, out of the powder, a smaller sample of 3 g was taken. Next, the powder was dried twice, at 130°C, for 2 hours (Drzazga 1999). The total amount of protein in the seeds was evaluated following the Kjeldhal method. To 0.5 g of the sample, a catalyst (3.5 g K_2SO_4 and 0.4 g $CuSO_4$) and H_2SO_4 were

added. The blind sample was done with 18 ml of H_2SO_4 and a tablet of the catalyst. Then, the sample was incinerated in an oven (Buchi), and mineralisation took place at 480°C for 1 hour. The sample was then titrated with 0.1 M HCl (Krełowska-Kułas 1993). Chemical analyses were made in triplicate.

Statistical data processing

The data obtained was processed by statistical analysis. The significant differences were calculated based on Duncan's test for $\alpha = 0.05$. For some chemical analyses data, standard deviation (SD) was calculated. The data for protein was calculated for dry mater (Nx6.25).

RESULTS AND DISCUSSION

All tested cultivars and breeding lines varied in terms of their emergence in the field (Tab. 2). The average values in the years 2011, 2012, were 75.9 and 29.9%, respectively. The best emergence was recorded in 2011, when the values ranged from 19.4 to 93.8%. Three lines were clearly outstanding: L4, J (Fig. 1) and C1 (Tab. 2). In 2012, they ranged from 4.6 to 53.8%. The seeds of three other lines: L1, J2 and J5, although emerged in the field, still did not develop ripe pods and seeds.

Fig. 1. Plants of the breeding line J with set pods and seeds. The pictures were taken in September, 2011

In 2011 and 2012, the number of pods per plant varied from 0 to 2.8 and from 0 to 6.2, respectively. Two lines: J and Z1, had the highest values of this character, while three others: L1, J2 and J5 did not form any pods and seeds. Although all the tested plants were blooming in August, some of them did not set pods, or set them but the seeds did not mature. In 2012, the average number of pods per plant increased to 2.91. Although the tested lines emerged and the plants set pods, still the set seeds were not matured and thereby there were no seeds for germination test and

chemical analyses. In 2011 and 2012, in terms of the number of pods per plant, 2 breeding lines were outstanding: J and Z1 (Tab. 2)

Table 2. Field emergence (%) and number of pods per plant in the selected adzuki bean cultivars and breeding lines

Cultivars and breeding lines	Field emergence (%)		Number of pods per plant	
	2011	2012	2011	2012
R	60.0 bc[1)]	37.5 a	2.0 a	0.2 a
B	90.6 de	35.4 a	2.0 a	6.2 e
J	91.3 de	20.9 a	2.8 b	4.2 cde
Z1	87.5 de	42.5 a	2.4 b	5.4 de
L1	88.8 de	50.0 a	0.0 a	0.0 a
L4	93.8 e	47.4 a	1.4 ab	5.0 de
L10	90.6 de	53.8 a	1.8 ab	4.8 de
J2	67.6 bc	8.2 a	0.0 a	0.0 a
J5	44.9 b	26.2 a	0.0 a	0.0 a
J6	72.5 c	27.2 a	1.2 ab	3.4 bcd
J7	19.4 a	4.6 a	1.0 a	4.8 de
J8	88.8 de	19.1 a	1.8 ab	1.8 ab
C1	91.3 de	15.5 a	2.0 abc	2.0 abc

[1)] Means followed by the same letters in the same column are not significantly different according to Duncan's test for $\alpha = 0.05$

In 2011, the number of seeds per pod varied from 0 to 6.2 (Tab. 3). Line B was the best in the study: it had more than 6 seeds per pod. The average (calculated for all the lines) was 4.36. Two lines: B and L10, were outstanding. In 2012, although the plants set pods, still they did not set seeds or the set seeds were not fully ripe when harvested

Among the seeds collected from the field in 2011, the germination capacity varied from 0 to 54% (Tab. 3). The average value was 32.07%. For 3 out of the 10 cultivars and breeding lines tested, the produced seeds did not germinate at all, whereas for 7 others, 5 germinated below 50%, whereas 2 breeding lines: C1 and L4, had germination above 50%.

In both years of the study, some variability was observed in the health status of the studied adzuki bean breeding materials. However, in general, all of them showed good health. During the entire growing season, not a single plant was removed from the research plot due to any damage done by pests or diseases. The average value of health for 2012 was higher than in 2011 (Tab. 4). In the test, good health status was characteristic of one and six objects, respectively, and in the second year – 4 and 6. A remarkable object was the line J5 which in both years reached the value of 4. Three objects: L1, L4 and J2 reached the value of 3 in the first and 4 in the second year.

Table 3. Number of seeds per pod and seed germination capacity (%) in selected adzuki bean cultivars and breeding lines

Cultivars and breeding lines	2011	
	Number seeds per pod	Seed germination capacity
R	4.3 b	0.0 a
B	6.2 c	0.0 a
J	3.9 b	49.0 cd
Z1	4.1 b	43.8 bcd
L1	–	–
L4	4.6 b	53.5 d
L10	5.0 bc	42.2 bc
J2	0.0 a	–
J5	–	–
J6	3.7 b	40.3 bc
J7	3.4 b	0.0 a
J8	4.1 b	37.9 b
C1	4.3 b	54.0 d

Explanations as in Table 2; "–" - not detected

The seeds of the tested cultivars and breeding lines varied in their total protein content (Tab. 5). In 2011, the total protein content in the seeds of the tested lines varied from 20.42 (L10) to 24.52% (J). Six lines: R, B, J and C1 had high contents of total proteins. The percentages of dry matter for all cultivars and lines tested were at a similar level of about 85%. In 2012, no chemical analyses of the seeds were done because no seeds were available.

Table 4. Health status of selected adzuki bean cultivars and breeding lines in the years 2011 and 2012

Cultivars and breeding lines	2011	2012
R	2*	1
B	2	4
J	3	3
Z1	3	2
L1	3	4
L4	3	4
L10	2	3
J2	3	4
J5	4	4
J6	2	3
J7	2	4
J8	3	3
C1	2	2

* 5-fully healthy, 1-fully contaminated

Table 5. Total protein (%) and dry matter (% d.m.) contents in the seeds of selected adzuki bean cultivars and breeding lines

Cultivars and breeding lines	2011[1)]	
	Total protein (% d.m.)	Dry matter (%)
R	21.842 ± 0.481	84.878 ± 0.012[1)]
B	21.515 ± 0.434	85.456 ± 0.027
J	24.22 ± 0.030	85.024 ± 0.010
Z1	21.365 ± 0.317	85.519 ± 0.114
L1	–	–
L4	20.834 ± 0.234	85.45 ± 0.057
L10	20.429 ± 0.059	85.775 ± 0.203
J2	–	–
J5	–	–
J6	21.221 ± 0.430	85.673 ± 0.007
J7	–	–
J8	21.635 ± 0.140	85.077 ± 0.119
C1	21.462 ± 0.403	85.028 ± 0.110

[1]In 2012, due to bad weather conditions, there were no seeds for chemical evaluation

The field studies and laboratory tests showed that out of 13 cultivars and breeding lines of adzuki bean, one line – marked J – proved to be the best (Fig. 1, Tab. 6). Slightly worse range was obtained for line Z1. Noteworthy are also three other lines: L4, J8 and C1. In Central Europe climatic conditions, they grew well, had good health status, high seed germination and high total proteins content (Tab. 6).

Table 6. Breeding summary of adzuki beans cultivars and breeding lines with the highest (marked with red colour) range in research field and laboratory experiments, and seeds characteristics in the years 2011-2012

Field emergence	Number of pods per plant	Number of seeds per pod	Seed germination	Good health status	High total protein content in seeds
B		B			
J	J		J	J	J
Z1	Z1		Z1	Z1	
L1				L1	
L4			L4	L4	
L10		L10	L10		
				J2	
				J5	
			J6		
J8			J8	J8	
C1	C1		C1		

In the last 30 years, in the European Union, a dynamic development of the seed sector has been observed. It refers not only to bigger seeds production and their trade, but also to new investments in plant breeding and in new breeding methods (Mumby 1994, Le Buanec 2000, Hołubowicz 2014). So far, in the seed business, we observe no symptoms of any crisis. As a result, new investments are being made in breeding to find new products for the market. These could be a new cultivar or a new vegetable species, not yet known to the client (Hołubowicz 2014). The results presented in this paper show that one of such new market products could be adzuki bean.

Grain legumes, including adzuki bean, are important sources of food in the vegetarian diet in developing countries. Their seeds contain about 2-3 times more protein as compared with cereals, however, their protein digestibility is very poor. This has been generally attributed to the presence of protease inhibitors and polyphenols (Satwadhar *et al.* 1981). The experiment carried out proved that in the climatic conditions of Central Europe it is possible to produce germinating seeds of adzuki bean. This is in agreement with earlier findings on growing the crop in Poland (Hołubowicz *et al.* 2009).

The tested lines varied in field emergence, number of pods per plant, number of seeds per pod, and plant health status. The big difference in the number of pods per plant, recorded for the years 2011 and 2012, could be explained by weather conditions. When growing the crop in the Central Europe climatic conditions, the optimal weather should include a dry and hot period in August, when the plants bloom, followed by a wet and warm period in September, when the set seeds fulfil and mature. This is still one more production proof that adzuki bean is a warm season crop (Sun *et al*. 2006).

The field observations clearly confirmed earlier reports of Gao *et al.* (2004) and Wu *et al.* (2011) that adzuki bean was a self-pollinated crop. No seeds with any evidence of unwanted cultivars and breeding line crosses were noticed. It is, though, obvious that future crossings of adzuki beans cultivars and breeding lines in breeding programmes will be required to create a new cultivar (Buishand 1956). In the case of a self-pollinated crop like adzuki bean, it will be easier to do and, therefore, also cheaper because no distance botanical isolation will be needed. It will lead to lowering breeding costs, which has been recently a common strategy in many seed companies (Kelly and Miklas 1998).

In the experiment, the number of seeds per pod in the tested lines was found to be stable. This characteristic can be used to determine breeding materials, especially as it is known that the germplasm pool of cultivated beans is very limited (Sullivan, 1988). However, this opinion does not follow the observations of common bean breeders (Korohoda 1969, Żuradzka and Angelus 1993).

Another positive information coming out from the research was no recorded damage on the seeds, done by bean weevil. All seeds collected from the field were healthy. This is in agreement with the information of Dobie *et al.* (1990) who reported 6 adzuki bean cultivars to be completely resistant to two bruchids: *Acanthoscelides obtecus* Say and *Zabrotes subfasciatus* Boheman. This could be due to the darker colour of adzuki bean seeds than that of the common bean (Bralewski *et al.* 2005).

Out of thirteen breeding lines and cultivars tested, two had germination capacity over 50%. This result is in agreement with earlier observations of adzuki bean seeds produced in Polish climatic conditions, reported by Hołubowicz *et al.* (2009).

The produced adzuki bean seeds had total protein content from 20.22 to 24.52%. It was lower than that described by Sun *et al.* (2006), but higher than described by Purseglove (1968). This could be related to different climatic conditions (Copeland and McDonald, 1995).

Out of the tested adzuki bean cultivars and breeding lines, two of them were the best in terms of their breeding and seed characters. The first one had high field emergence rate and high protein content. The latter had a little lower protein content than the first one.

CONCLUSIONS

In cold and wet years, when grown in Poland, the adzuki plants did not set up seeds. In years with favourable weather conditions, the produced seeds had germination capacity from 0 to 54%. The evaluated cultivars and breeding lines varied in terms of field emergence, earliness, number of pods per plant, number of seeds per pod, and plant health status. The harvested seeds had no symptoms of damage by bean weevil. They had from 20.49 to 25.22% d.m. of total proteins and there was no difference in their dry matter content.

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WYBRANE CECHY HODOWLANE I ZAWARTOŚĆ BIAŁKA OGÓLNEGO W NASIONACH RÓŻNYCH ODMIAN FASOLI ADZUKI (*PHASEOLUS ANGULARIS* W.H. WHITE) UPRAWIANEJ W ŚRODKOWEJ EUROPIE

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Streszczenie. W latach 2011-2012 trzynaście odmian i linii hodowlanych fasoli adzuki (*Phaseolus angularis* W.H. White) pochodzenia chińskiego, uprawianych w warunkach klimatycznych Europy Środkowej, było oceniane pod względem wybranych cech hodowlanych i zawartości białka ogółem w nasionach. W doświadczeniu polowym oceniano wschody, liczbę łuszczyn na

roślinie, liczbę nasion w łuszczynie i stan zdrowotny roślin. Natomiast badania laboratoryjne obejmowały zdolność kiełkowania nasion, suchą masę i ogólną zawartość białka. Produkcja nasion fasoli adzuki w zachodniej Polsce była możliwa. Jednak w zimnym i wilgotnym roku 2012 nasion nie udało się pozyskać. Badane materiały różniły się we wczesności wschodów, liczbie łuszczyn na roślinie, liczbie nasion w łuszczynie i stanem zdrowotnym roślin. Wytworzone nasiona miały zdolność kiełkowania od 0 do 54%. Zawartość białka ogółem w pozyskanych nasionach wyniosła od 20,01 do 31,91% s.m., więc mogą one stanowić dodatkowe źródło cennego białka roślinnego. Wśród ocenianych odmian i linii hodowlanych jedna linia hodowlana (oznaczone literą J) okazała się najlepsza, natomiast druga (Z1) była również interesująca pod względem ocenianych w doświadczeniach cech hodowlanych.

S ł o w a k l u c z o w e: fasola adzuki, *Vigna angularis*, cechy hodowlane, kiełkowanie nasion, białko