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Physiological and Agro-biological Traits Evaluation of Several Local Grain Legumes under Climatic Condition of South-central Region of Bulgaria

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Abstract. The most important cool season grain legumes (dry pea, chickpea, broad bean, lentil, lupins, grass pea and common vetch) are widely grown in many parts of the world. The aim of the current study was to test some of them under the drought-prone area of Sadovo (south-central region of Bulgaria) and to evaluate their adaptability based on physiological and agro-biological traits. The investigation was carried out at the experimental field of IPGR including several local accessions of white lupin (*Lupinus albus* L.), chickpea (*Cicer arietinum* L.) and grass pea (*Lathyrus sativum* L.). The chlorophyll content index of the leaves, used as criteria for drought tolerance, was measured by portable CCM 200 plus- Chlorophyll Content Meter. The assessment of agro-biological traits was performed according to the International Descriptor for each crop. Under the drought stress conditions the highest value of the chlorophyll content index was observed in three lupin accessions (BGR 6341, BGR3080, BGR3085), in two from chickpea (BGR 40417 and B9E0149) and in four from grass pea (BGR 40415, BGR 4835, BGR4847, BGR4834). The highest yield potential was established in two lupin (BGR 6341 and BGR 3084), two chickpea accessions (BGR 23151 and B9E0149) and three grass pea (BGR4832 and BGR40415). The selected accessions were included in the list for further investigations concerning drought tolerance and maintenance of relatively high yield potential under drought stress conditions.

Key words: lupin, chickpea, grass pea, drought tolerance, agro-biological evaluation.

Introduction

Legumes are unique plants, with great value for agriculture and society because of that they contribute to many different functions and ecosystems services (Nemecek et al., 2008; De Faria et al., 2011). One approach that can contribute to mitigating climate change is including legume food, forage, and tree crops in farming systems (Jensen et al., 2012). Grain legumes crop have positive influence on global food and nutrition security. However, their production rate remains unsatisfying compared to their consumption rate due to biotic and abiotic stress factors (Ojiewo et al., 2018). Water deficit is one of the most important factors that not only affect plant growth and development but also limit productivity (Boyer, 1982; Choudhary & Suri, 2014). Under the stress of extreme drought, grass pea is the only productive crop and becomes the only food for the poor in some rural or marginal areas (Vaz Patto et al., 2006). Terminal drought still limits chickpea production and grain yield although, chickpea is considered as drought-tolerant, cool-season food legume. The seed yield can be reduced by 58−95% compared to irrigated crops due to terminal drought. Drought stress causing reduction in pod production and abortion (the chief factors affecting the overall grain yield) (Leport et al., 2006). The limiting factor in the development and yield of plants is strongly reduced water content in soil. It was observed the highest reduction of seed yield, as a result of drought occurring in the flowering period, in blue lupine, cultivated both in pure sowing and in mixture with barley (Podleśny & Podleśna, 2010). Synthesis of plastid pigments is of significant importance for the photosynthetic activity of plants. The photosynthetic pigments are one of internal factors which could limit the photosynthetic activity to a large extend. Their content in normal and stress environmental conditions has been widely studied and discussed (Mikiciuk et al., 2010; Wrobel et al., 2010; Aienl et al., 2011). It is proven that the reduction of the pigment concentration is an indicator of stress in cases as water and temperature stress, insufficiency or excess of mineral elements, etc. (Hendry & Grime, 1993; Stoeva et al., 2010). A deficiency of water will additionally disturb effects such as water relations, membrane integrity, yield, development, osmotic adjustment, photosynthetic movement and pigment content (Benjamin & Nielsen, 2006; Kaur & Kumar, 2020). Notably, due to the severe pressure of global climate change and ever increasing demand for food production, implementation of high-throughput and cost-effective techniques is required, which would invariably support the traditional breeding schemes. Therefore, immediate attention needs to be placed towards large scale exploration and characterization of the available germplasm for abiotic stresses tolerance (Jha et al., 2014).

The aim of the current study was to assess the drought tolerance of several local accessions of white lupin (*Lupinus albus* L.), chickpea (*Cicer arietinum* L.) and grass pea (*Lathyrus sativum* L.) by their physiological and agro-biological traits under the drought-prone area of Sadovo.

Material and Methods

Two years (2019-2020) investigations were carried out on the experimental field of Institute of Plant Genetic Resources (IPGR) - Sadovo. The plants were grown by standard technology for field production on cinnamon-forest soils after a wheat precursor.

The subjects of the study were six white lupin accessions, six – chickpea and eleven local grass-pea. The seeds were obtained from the grain legums collections, stored in the IPGR gene-bank. Every one accession was sown in 5 to 10 rows (depending on number of seeds), at a depth of 4-6 cm and a distance between the rows of 30-50 cm.

The physiological assessment of the all studied accessions of lupin, chickpea and grass pea was carried out in the field. The plants were evaluated twice at two stages - beginning of flowering and end of flowering/beginning of ripening. Samples collection was performed during two periods of drought: end of April/May, 2019 and May/beginning of June, 2020. The temperature and precipitation sum during the periods of measurements are presented in Table 1. The precipitation during the first and second date of measurements in two years investigations was low (7.8 l/m^2 - 12.1 l/m^2; 12.6 l/m^2 - 14.1 l/m^2, respectively).

Table 1. Temperature conditions and precipitation sum in May and June during the period 2019–2020.

Year	**2019**				**2020**			
Parameter	**Average temperature, (t°)**		**Precipitation (l/m^2)**		**Average temperature, (t°)**		**Precipitation (l/m^2)**	
Month	V	VI	V	VI	V	VI	V	VI
1-10 days	15.83	20.92	7.80	135.00	17.01	19.96	12.60	12.00
11-20 days	18.18	25.46	3.60	32.10	21.62	21.70	0.00	19.80
20-30 days	20.94	24. 99	5.90	12.10	15.54	23.25	27.50	14.10

The relative chlorophyll content, expressed by chlorophyll content index (CCI) in the leaves, was measured with Chlorophyll Content Meter (CCM 200 plus). The measurements were carried out on 20 leaves per accession. This apparatus is ideal for investigation of crop stress, leaf senescence, health determination. Furthermore, the affordability and ease of use make it an exceptional teaching tool for botany and plant science courses (Richardson et al., 2002; Arunyanark et al., 2008).

The structural elements of the yield were established by biometric analysis of 10 plants per accession. The traits evaluation was performed according to the descriptors for *Lathyrus* spp. (IPGRI, 2000), *Cicer arietinum* (UPOV, 2019) and *Lupinus* sp. (UPOV, 2004). The following quantitative characters were taken into consideration: plant height (cm), number of productive branches, height to the first pod (cm), number of pods per plant, number of grains per plant, number of grains per pods, mass of grains per plant (g) and mass of 100 grains (g). The length of vegetation period comprised the days from plant germination to 80% ripening of plants.

The analysis of variance and descriptive statistics (Mean, Error of mean, Minimum, Maximum, Range, Standard deviation and Coefficient of variation) were made with statistical program SPSS 19.0 for Windows. The means were compared by the three significance difference (LSD) at the 0.05, 0.01 and 0.001 probability level (p). The degree of variability of the studied traits, represented by a coefficient of variation (CV, %) was indicated according to the scheme of Mamaev (Shamov, 1998): up to 7% - very low; 7.1-12% - low; 12.1-20% - average; 20.1-40% - high; over 40% - very high.

Results and Discussion

Lupin (*Lupinus albus* L.)

Evaluation of the relative chlorophyll content, expressed by chlorophyll content index (CCI) in six white lupin accessions

The CCI (mean value of two years) during the first date of measurement in lupin accessions varied from 6.34 (BGR3086) to 14.01 (BGR3080) and they were significantly different (p=0.05), compared to the mean standard (Table 2). During the second date of measurement, CCI mean values in all analyzed accessions decreased considerably and high variation was not observed between the accessions – from 3.30 (B9E0202) to 5.88 (BGR6341). In some accessions, the CCI value dropped with 76.0% for BGR 3080 but only with 12.0% for BGR 3086 compared to the first date of measurement. The lupin accessions had high variation (29.3%) of the CCI values during the first measurement dates and average variation (18.9%) during the second date.

It is worth to pay attention on BGR 3080, BGR 3085 and BGR 6341 accessions characterized with high CCI during the first date of evaluation under the relatively low rate of precipitations.

Table 2. Mean value of Chlorophyll Content Index of six white lupin accessions in two years trial (2019–2020). Legend: n.s. no significance difference.

Accessions	Chlorophyll content index (first date)	Chlorophyll content index (second date)	Chlorophyll content index (mean)
B9E0202	8.14 $^{n.s.}$	3.30 $^{n.s.}$	5.72
BGR3084	9.08 $^{n.s.}$	5.01 $^{n.s.}$	7.05 $^{n.s.}$
BGR3080	14.01*	5.04 $^{n.s.}$	9.52 $^{n.s.}$
BGR3085	10.82 $^{n.s.}$	5.88 $^{n.s.}$	8.35 $^{n.s.}$
BGR3086	6.34*	5.57 $^{n.s.}$	5.95 $^{n.s.}$
BGR6341	13.37 $^{n.s.}$	5.88 $^{n.s.}$	9.62 $^{n.s.}$
X-mean standard	10.29	5.11	7.70
Min	6.34	3.30	5.72
Max	14.01	5.88	9.62
CV (%)	29.3	18.9	22.4
p=0.05 *	3.35	2.96	2.32
p=0.01 **	4.47	3.94	3.08
p=0.001 ***	5.83	5.15	3.97

Descriptive statistics of quantitative traits in six white lupin accessions

High variation of almost all quantitative traits were observed in white lupin accessions except the vegetation period (CV-3.89%) and height to the first pod (CV-10.85%) (Table 3). Very high value of coefficient of variation (over 40.0%) were established between accessions in the following traits: mass of grains per plant, number of grains per plant, number of productive branches and number of pods per plant.

Several genotypes were selected based on their valuable traits. Two accessions (BGR 6341 and BGR 3084) had the biggest mass of grains per plant. They also had a big number of pods and grains per plant and big number of grains per pod.

The earliest accessions with shortest vegetation period were – BGR 3086, BGR 3080 and B9E0209. All genotypes of white lupin had vegetation period between 90 and 99 days.

Table 3. Evaluation of quantitative traits in six white lupin accessions in two years trial (2019-2020).

Characteristics	Range	Min	Max	Mean		Std. Deviation	Variance	CV (%)
				Statistic	Std. Error			
Plant height (cm)	37.10	30.33	67.43	48.13	5.03	12.33	152.07	25.62
Height to the first pod (cm)	8.40	24.17	32.57	29.10	1.29	3.15	9.95	10.85
Number of productive branches	4.26	0.17	4.43	1.82	0.66	1.61	2.60	88.72
Number of pods per plant	12.97	2.17	15.14	6.23	1.91	4.68	21.95	75.28
Number of grains per plant	59.26	5.17	64.43	20.73	9.14	22.39	501.49	108.01
Number of grains per pods	2.95	2.08	5.03	3.11	0.46	1.13	1.27	36.16
Mass of grains per plant (g)	21.32	0.88	22.20	6.50	3.30	8.08	65.34	124.38
Mass of 100 grains (g)	13.25	23.50	36.75	30.01	2.56	5.73	32.85	44.26
Vegetation period (days)	10.00	91.00	101.00	94.33	1.50	3.67	13.47	3.89

The combined analysis of variance revealed significant differences among genotypes for all studied traits indicating wide genetic variation among genotypes and this provides basis for selection among these genotypes. Our study is consistent with those of Abo-Hegazy et al. (2020). Abo-Hegazy et al. (2020) who also reported for significant differences among tested accessions by the most important quantitative traits.

Promising white lupin accessions were selected by carried out screening under drought stress conditions, based on their CCI and quantitative traits. BGR 6341, BGR3080 and BGR3085 possessed the highest value of the chlorophyll content index in the first date of measurement. The genotype BGR 3080 was distinguished with tall plants (63.86 cm) and large grains (40.80 g) while BGR 3085 had big number of pods and grains per plant (14.43 and 60.14, respectively) and high yield per plant (22.14 g). The genotype BGR 6341 had tall plants (67.43 cm), big number of productive branches (4.43), high values of number of pods per plant (15.14), number of grains per plant (64.43), number of grains per pod (5.03) and large grains (22.20 g). These accessions can be used as an initial gene sources in the breeding program.

The lowest reduction of the CCI between the first and second date of assessment in BGR 3086 accession could be considered as type of drought stress tolerance. This accession characterized also with a big number of grains per plant (52.86), tall plants (68.43 cm) and the shortest vegetation period (90 days), which is a reason to be selected as gene source. Similar results were reported by Juson et al. (2019), in a study of drought resistance in yellow lupin and pea cultivars.

Chickpea (*Cicer arietinum* L.)

Evaluation of the relative chlorophyll content, expressed by chlorophyll content index (CCI) in six chickpea accessions

The CCI (mean value of two years) during the first date of measurement in chickpea accessions varied from 2.72 (BGR23151) to 5.10 (BGR40417) and the first one was significantly different (p=0.05), compared to the standard (Table 4). Three genotypes (BGR23151, A9E0121 and A8E0412) had low CCI values with significance difference compared to standard during the first measurement date. During the second date of measurement, CCI mean values in all analyzed accessions decreased weakly and high variation was not observed between the accessions – from 2.80 (A8E0412) to 5.11 (B9E0149). Two accessions - BGR 23151 and A8E0412 had low mean values of CCI with significantly difference compared to standard. Two other accessions (BGR40417 and B9E0149) had the highest and stable values of CCI during the first and second date of measurements. The chickpea accessions had high variation of the CCI values during the first and second measurement dates (27.9% and 23.8% respectively).

Table 4. Chlorophyll Content Index of six chickpea accessions in two years trial (2019-2020). *Legend:* n.s. no significance difference.

Accessions	Chlorophyll content index (first date)	Chlorophyll content index (second date)	Chlorophyll content index (mean)
St. BGR 40417	5.10	4.46	4.78
BGR 23151	2.72*	3.08 $^{n.s.}$	2.90 *
B9E0001	3.31 $^{n.s.}$	4.19 $^{n.s.}$	3.75 $^{n.s.}$
B9E0149	5.34 $^{n.s.}$	5.11 $^{n.s.}$	5.22 $^{n.s.}$
A8E0412	2.97*	2.80 $^{n.s.}$	2.89 *
A9E0121	2.88*	5.05 $^{n.s.}$	3.97 $^{n.s.}$
X	3.72	4.11	3.92
Min	2.72	2.80	2.89
Max	5.34	5.11	5.22
CV (%)	27.9	23.8	24.4
p=0.05 *	1.88	2.10	1.54
p=0.01 **	2.50	3.14	2.04
p=0.001 ***	3.26	4.07	2.62

Descriptive statistics of quantitative traits in six chickpea accessions

High value of coefficient of variation was observed in number of pods and grains per plant (VC-41.83% and 43.31% respectively) in tested chickpea accessions (Table 5). There was a small difference among accessions in plant hight, number of grains per pod and vegetation period. These characters were slightly variable with coefficient of variation up to 12%.

Two accessions (BGR23151 and A9E0149) had big number of pods (58.17 and 59.25, respectively) and grains per plant (51.33 and 52.50, respectively). They also had tall plants-44.33 cm and 49.17 cm, respectively.

The vegetation period for all chickpea accessions varied between 83 and 107 days. The shortest vegetation period was established in following accessions: A8E0412 (83 days), A9E0121 (87 days) and A9E0149 (88 days).

Table 5. Qualitative traits observed on six chickpea accessions in two years trial (2019-2020).

Characteristics	Range	Min	Max	Mean Statistic	Mean Std. Error	Std. Deviation	Variance	CV (%)
Plant height (cm)	9.17	40.00	49.17	43.75	1.31	3.22	10.36	7.40
Height to the first pod (cm)	12.00	16.83	28.83	20.76	1.75	4.28	18.31	22.35
Number of prodoctive branches	1.92	1.08	3.00	2.15	0.27	0.65	0.43	27.55
Number of pods per plant	36.00	22.17	58.17	35.14	6.38	15.62	244.04	41.83
Number of grains per plant	39.08	20.17	59.25	35.67	6.66	16.31	265.91	43.31
Number of grains per pods	0.23	1.00	1.23	1.10	0.03	0.08	0.01	7.06
Mass of grains per plant (g)	13.68	9.55	23.23	13.98	2.21	5.41	29.25	36.39
Mass of 100 grains (g)	15.25	37.00	52.25	42.54	3.01	7.37	54.30	17.00
Vegetation period (days)	24.00	83.00	107.00	94.33	3.93	9.63	92.67	10.37

Mafakheri et al. (2010) and Maqbool (2017) reported significant variability in chickpea germplasm as a response to drought stress expressed as drought escape, drought avoidance and drought tolerance. These mechanisms prevent chickpea crop from harmful effects of drought. In our study promising chickpea accessions were selected under drought stress conditions, based on their CCI and quantitative traits. BGR 40417 and B9E0149 possessed the highest value of the chlorophyll content index during the first and second date of measurement. The same accessions possessed also some other valuable traits which could be interesting from breeding point of view. The genotype BGR40417 formed first pods high (28.83 cm) while accessions B9E0149 had tall plants (49.17 cm) and big number of pods and grains per plant (51.33 and 52.50, respectively). BGR 40417 and B9E0149 accessions will be used as initial gene sources in breeding program. The slight variation observed in our study in several quantitative traits is in contrast to Petrova & Angelova (2011) and Petrova & Stamatov (2013) who established wide genetic variation in agro-morphological traits of evaluated chickpea collection. This fact could be explained with the smaller number of tested accessions in our study.

Grass pea (*Lathyrus sativus* L.)

Evaluation of the relative chlorophyll content, expressed by chlorophyll content index (CCI) in eleven grass pea accessions

The CCI (mean value of two years) during the first date of measurement in grass pea accessions varied from 9.55 (BGR4833) to 16.78 (BGR40415) and the second one was significantly different (p=0.05), compared to the mean standard (Table 6). During the second date of measurement, CCI mean values in all analyzed accessions decreased considerably and not high variation was observed between the accessions – from 1.63 (BGR33111) to 2.83 (BGR4830). All accessions showed strong decrease in CCI values, an average to 80.00%, during the second measurement date. All studied genotypes showed no significance difference in CCI values during the second measurement date (Table 5). The grass pea accessions had average variation of the CCI values during the first and second measurement dates (16.10% and 19.40% respectively).

Descriptive statistics of quantitative traits in eleven grass pea accessions

The grass pea accessions had low variation of the most of studied traits – vegetation period (2.44%), number of grains per plant (9.02%), mass of 100 grains (9.53%) and plant height (11.89%) (Table 7). Big differences were observed in the mass of grains per plant, number of grians per plant and number of pods per plant. These characters were the most variable with high value of coefficient of variation. The vegetation period varied between 106 and 115 days. The earliest accession, with the shortest vegetation period, was BGR 4334 (106 days) and the latest one – BGR4833 (115 days). Those two genotypes had tall plants, with high first formed pods and large grains. The accession BGR4832 had the highest number of pods and grains per plant and mass of grains per plants while genotype BGR 4847 had the lowest one.

Table 6. Chlorophyll Content Index of eleven grass pea accessions in two years trial (2019-2020). Legend: n.s. - no significance difference.

Accessions	Chlorophyll content index (first date)	Chlorophyll content index (second date)	Chlorophyll content index (mean)
BGR40415	16.78*	2.41 n.s.	9.59*
BGR4830	10.14 n.s.	2.83 n.s.	6.48 n.s.
BGR4831	11.55 n.s.	2.35 n.s.	6.95 n.s.
BGR4832	10.19 n.s.	1.99 n.s.	6.09 n.s.
BGR4833	9.55 n.s.	2.26 n.s.	5.91 n.s.
BGR4834	11.52 n.s.	2.81 n.s.	7.16 n.s.
BGR4835	12.53 n.s.	2.69 n.s.	7.61 n.s.
BGR4836	11.66 n.s.	3.09 n.s.	7.38 n.s.
BGR4847	13.43 n.s.	1.88 n.s.	7.66 n.s.
BGR33111	11.81 n.s.	1.63 n.s.	6.72 n.s.
BGR43334	12.48 n.s.	1.92 n.s.	7.20 n.s.
X-mean standard	11.87	2.35	7.16
Min	9.55	1.63	9.59
Max	16.78	3.09	5.16
CV (%)	16.1	19.4	13.8
P=0.5 *	4.19	0.89	1.96
P=0.01 **	5.53	1.17	2.58
P= 0.001 ***	7.11	1.51	3.31

Table 7. Qualitative traits observed on eleven grasspea accessions in two years trial (2019–2020).

Characteristics	Range	Min	Max	Mean		Std. Deviation	Variance	CV (%)
				Statistic	Std. Error			
Plant height (cm)	28.17	54.83	83.00	70.11	2.51	8.34	69.53	11.89
Height to the first pod (cm)	15.50	19.50	35.00	25.08	1.28	4.25	18.04	16.94
Number of prodoctive branches	1.67	3.00	4.67	3.65	0.14	0.46	0.21	12.66
Number of pods per plant	17.33	16.67	34.00	23.70	1.69	5.59	31.23	23.58
Number of grains per plant	33.58	42.17	75.75	55.31	3.64	12.09	146.11	21.85
Number of grains per pods	0.74	2.43	3.17	2.69	0.07	0.24	0.06	9.02
Mass of grains per plant (g)	6.15	7.68	13.83	10.25	0.62	2.05	4.21	20.01
Mass of 100 grains (g)	5.60	18.10	23.70	20.79	0.60	1.98	3.92	9.53
Vegetation period (days)	9.00	106.00	115.00	110.55	0.81	2.70	7.27	2.44

According Donskoy (2013) the highest seed productivity in grass pea is determined by the weight of the plant, the number of pods per plant and the weight of the pods per plant. The efforts of researchers should be directed in growing the grass pea mainly for grain (Polignano et al., 2005).

During the current study promising grass pea accessions were selected, based on their CCI and quantitative traits expressed under drought stress conditions. BGR 40415, BGR 4835, BGR4847 and BGR4834 possessed the highest value of the chlorophyll content index in the first date and mean for the two date of measurement. These accessions differentiated also with valuable yield characters as: BGR40415 had big number of pods and grains per plant (29.75 and 75.75, respectively); BGR4835 – with tall plants (67.00 cm) and large grains (23.00 g); BGR4847 – with high first formed pod (35.00 cm) and big number of grains per pod (3.17) and BGR4834 – with plant height (64.83 cm) above the average standard and relatively short vegetation period (112 days). In similar study, Rybinski et al. (2008) reported average grain productivity between 7.20 g to 21.40 g in 106 grass pea lines. The selected accessions will be considered as starting materials for breeding.

Conclusions

Several local accessions of white lupin (*Lupinus albus* L.), chickpea (*Cicer arietinum* L.) and grass pea (*Lathyrus sativus* L.) were selected under the drought stress conditions of Sadovo region and based on their quantitative traits. Three white lupin accessions (BGR 6341, BGR3080, BGR3085) were selected as drought tolerant and with relatively high yield potential. Another lupin genotype (BGR 3086) had the lowest decrease of the CCI between the both dates of assessment, which could be considered as type of drought stress tolerance. Two promising chickpea accessions (BGR 40417 and B9E0149) were selected under the same environment as drought tolerant. The same accessions possessed also some other valuable traits which could be interesting from breeding point of view. Four grass pea accessions (BGR 40415, BGR 4835, BGR4847 and BGR4834) possessed drought tolerance and valuable yield characters. The selected accessions can be included in the list for further investigations concerning drought tolerance and maintenance of relatively high yield potential under drought stress conditions.

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