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SELECTION AT ULTRA-LOW DENSITY OF SECOND GENERATION LINES OF BEAN CULTIVARS UNDER WATER STRESS

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ABSTRACT

Selection in the absence of competition under ultra-low density has been proposed as a mean to develop new cultivars serving the needs of a sustainable agriculture under diverse climatic conditions. In 2018, individual high-yielding plants of the determinate type bean varieties (*Phaseolus vulgaris* L.) Iro and Pirgetos were selected in R21 honeycomb design trials under normal and deficit irrigation treatments. This led to 14 high-yielding second generation lines from cultivar Iro and four from cultivar Pirgetos which together with the original varieties were evaluated again in 2019 under the same ultra-low density and irrigation conditions. Water stress affected total chlorophyll content measured in selected individual plants from start of flowering until physiological maturity with some of the second generation lines showing similar or higher chlorophyll concentrations from the original varieties especially during the seed filling stage. Significant differences between normal and deficit irrigation were also shown on CO_2 assimilation rate for all genotypes, but there were no significant differences between the evaluated progenies and their respective ancestors. Compared to the original variety Iro, four of the second generation lines had similar or higher yield plant^{-1} (up to 15%) and exhibited the highest stability under both normal and water deficit conditions, whereas for the variety Pirgetos two progenies outperformed the original cultivar (by 5,5 to 27%). Further research is under way for a final evaluation of the selected progenies under farming density and water stress.

Keywords: *Ultra-low plant density, Water stress, Chlorophyll concentration,* CO_2 *assimilation rate.*

INTRODUCTION

Common bean (*Phaseolus vulgaris* L) is one of the most significant legume crops for human consumption due to its high protein content, fiber and other essential minerals (Graham and Ranalli, 1997). In Greece, beans are the most important pulses with increased cultivated areas in recent years (Kargiotidou *et al.,* 2019). They are cultivated during spring and summer and the most prominent area is in the north of Greece, specifically at relatively high altitudes and cool temperatures. Modern agriculture depends by far on uniform crop varieties in order to meet a growing demand for food by the world's population, and in most cases several landraces have progressively been replaced by elite cultivars satisfying the farmers and consumer's needs (Mavromatis *et al.,* 2007). The existence of genetic heterogeneity in Greek genotypes is offered for plant selection with new breeding strategies and main criterion plant yield while such germplasms could broaden the genetic base of commercial beans for developing high-yielding varieties with an improved performance under different environmental constraints (Papadopoulos *et al.,* 2007).

Honeycomb breeding has been suggested as an effective technique to exploit the genetic variability of gene pools like heterogeneous landraces or even early released varieties (Tokatlidis, 2015). This has been experimentally validated in single-plant progeny lines derived from two dry bean landraces of indeterminate type that were proved to be tolerant to heat stress and improved in yield capacity up to 38% (Tokatlidis *et al.,* 2010), as well as in a lentil landrace grown under low-input conditions (Kargiotidou *et al.,* 2014).

Water scarcity raises a soaring concern in the Mediterranean region, as higher temperatures and more frequent drought events are projected to occur due to climate change (Cammarano *et al.*, 2019). Water deficit is a major limiting factor for crop productivity worldwide resulting in significant seed yield reductions across 60% of global bean production areas (Soureshjani *et al*., 2019). Reduced water availability affects plant physiological responses resulting in a reduction in photosynthesis and transpiration rate, intercellular carbon dioxide concentration and activates stomatal closure by the accumulation of abscisic acid (ABA) causing growth inhibition and reduced plant productivity (Mathobo *et al.,* 2017; Soureshjani *et al.,* 2019). Chlorophyll content of common bean is also reduced as a result of the degradation caused by drought conditions (Beede *et al*., 2013), and is directly related to biomass accumulation. These responses depend on the intensity of the stress, the plant genotype, and the plant developmental stage at stress incidence, among other factors (Beebe *et al.,* 2013). The necessity to tackle this challenge has led to breeding and developing new varieties adapted to a continuously changing environment either exploiting intraspecific variability or by transferring genes from closely related wild species adapted to low irrigation (Martinez *et al.,* 2007). Extensive evidence exists to show that genetic resources for drought tolerance have potential for breeder programs (Andrade *et al.,* 2016; Farooq *et al.,* 2017).

The main objective of the present study was to evaluate high-yielding second generation lines of two bean varieties selected at ultra-low density under water deficit conditions during anthesis and seed-filling developmental stage. The range of variation in agronomic and physiological parameters that could exist would be utilized in further experiments under farmer's densities for identifying and developing improved genotypes which could perform better under adverse conditions.

MATERIALS AND METHODS

Plant material and experimentation

In 2018, individual high-yielding plants of the determinate type bean varieties (*Phaseolus vulgaris* L.) Iro and Pirgetos, developed originally by the Hellenic Industrial and Fodder Crops Institute, were selected in R21 honeycomb design trials under normal and deficit irrigation treatments in the main farm of the University of Western Macedonia in Florina as described in previous work (Papathanasiou *et al*., 2019). This led to 14 high-yielding second generation lines from cultivar Iro (coded IR1 to IR14) and four from cultivar Pirgetos (coded PIR1 to PIR4) which together with the original varieties and an imported one Great-northern type (GNTY) were evaluated again in 2019 under the same ultra-low density and irrigation conditions. Approximately 50 plants per second generation line were assessed in two R21 honeycomb design trials under normal and deficit irrigation treatments respectively. The experiments were sown on 11^{h} of May in the experimental farm of the University of W. Macedonia in Florina Greece (40°46′ N, 21°22′E, 707 m asl), in a sandy loam soil with pH 6.5, organic matter content 14.0 g kg^{-1}, N-NO_3 100 mg kg^{-1}, P (Olsen) 50.3 mg kg^{-1} and K 308 mg kg^{-1} and water holding capacity 21.8% (0 to 30 cm depth). The ultra-low density of 1.2 plants/m^2 was used i.e. single-plant hills were spaced 100 x 100 cm apart. Two or three seeds were sown in each hill and later thinned to obtain single-plant hills. A total of 400 Kg/ha 0-20-0 and 200 Kg/ha 11-15-15 fertilizers were applied at planting, while additional N (50 g per plant of a 27-0-0 fertilizer) was top-dressed when plants had reached the appropriate developmental stage. Complete weed control was obtained by tilling and hand.

Irrigation treatments

The normal irrigation received a full irrigation treatment, while deficit irrigation was 50% of the normal to simulate drought stress. The irrigation treatments are fully described in Papathanasiou *et al.,* (2019).

Chlorophyll and gas-exchange measurements

Total chlorophyll content was measured with a hand-held dual-wavelength meter (SPAD 502, Chlorophyll meter, Minolta Ltd., Japan) at five 10-day intervals from start of anthesis until physiological maturity (SPAD1 to SPAD5) in six plants of each genotype in normal and deficit irrigation conditions. A portable photosynthesis system that measures CO_2 uptake (LI-6400 XT, Li-Cor, USA) equipped with a square (6.25 cm^2) chamber was used for determinations of CO_2 assimilation rate (A), transpiration rate (E) and stomatal conductance to water vapour (g_s) during the seed filling period. Leaf gas exchange was measured in the middle leaflet of a fully expanded trifoliate leaf close to the top of the plants. Measurements were performed on the same six plants of each genotype that chlorophyll measurements were taken from 09:00-12:00 in the morning to avoid high vapor-pressure deficit and photoinhibition at midday.

Harvest and statistical analysis

Plants were harvested individually and seed yield was measured at the physiological maturity stage and recorded at a per-plant basis for both normal and deficit irrigation treatments. Comparison of means was conducted by Least Significance Difference Test (LSD) after analysis of Variance (ANOVA), for completely random design.

RESULTS AND DISCUSSION

Grain yield plant^{-1}, change% compared to the original genotype and stability% at ultra-low density under normal and deficit irrigation for the eighteen second generation lines and the controls are presented in Table 1. Deficit irrigation significantly reduced the yield plant^{-1} in all the second generation lines and their original genotypes but with different intensity. In the normal irrigation conditions the genotypes IR1 with 134,2 g yield plant^{-1}, IR8 with 159,2 g and an increase compared to the control of 14,2%, IR7 with 136,2 g and 100% stability and the genotype IR2 with 139,4 g exactly the same yield as the original genotype Iro and 80,5% stability where the ones that stood out. Under the water stress conditions the 2nd generation lines that outperformed the original variety Iro in terms of yield plant^{-1} were the genotypes IR12 with 63,2 g and an increase compared to the control 15,5% while its stability ranged at 94,0%, the IR4 with 60,5 g and 10,6% increase in yield plant^{-1}, IR8 with 60,4 g, 10,5% increase and stability 83,7%, IR2 with a yield of 56,1 g, IR1 with 55,8 g, and also the genotype IR7 which had a yield plant^{-1} of 55,5 g, higher by 1,5% compared to the yield of the original variety and stability 82,8%. Considering the behavior of the 2nd generation lines originating from the variety Iro in both irrigation treatments we see that the genotypes IR1, IR2, IR7 and IR8, stood out for further evaluation. Similarly, the 2nd generation lines that outperformed the original variety Pirgetos either in yield plant^{-1} or in stability where the genotype PIR1 with 159,2 g and an increase of 27% compared to the control in normal irrigation and 67,8 g, higher 8% than the original variety under deficit irrigation and the genotype PIR3 with a yield plant^{-1} of 119,1 g and a high stability of 97,8% under the normal irrigation conditions and 66,2 g, higher by 5.5%

compared to the yield of the original variety Pirgetos and 100% stability under the water stress conditions (Table 1). This is in agreement with other studies where under adverse conditions such as high temperatures and increased biotic stress second generation sister lines of bean and/or other legumes such as lentils, outperformed the original genotypes under ultra-low density (Papadopoulos *et al.*, 2004; Kargiotidou *et al.*, 2014; Vlahostergios *et al*., 2018).

Table 1. Grain yield plant^{-1} (g), change compared to the original genotype (Change%) and stability (%) at ultra-low density under normal and deficit irrigation for the eighteen second generation lines and the controls evaluated.

	Normal Irrigation			**Deficit Irrigation**		
Second generation lines	**Yield g plant^{-1}**	**Change %**	**Stability %**	**Yield g plant^{-1}**	**Change %**	**Stability %**
IR-Control	**139,4**		**79,0**	**54,7**		**75,6**
IR1	134,2	-3,7	94,1	55,8	+2,0	83,4
IR2	139,4	0,0	80,5	56,1	+2,5	78,0
IR3	112,9	-19,0	82,1	43,8	-19,9	82,2
IR4	129,9	-6,8	69,4	60,5	+10,6	84,1
IR5	101,5	-27,2	81,9	43,3	-20,9	64,6
IR6	113,8	-18,4	67,3	43,9	-19,7	82,3
IR7	136,2	-2,3	100	55,5	+1,5	82,8
IR8	159,2	+14,2	80,5	60,4	+10,5	83,7
IR9	118,2	-15,2	91,8	52,3	-4,3	93,1
IR10	130,4	-6,4	79,1	54,4	-0,6	80,3
IR11	133,7	-4,1	74,1	54,6	-0,2	65,3
IR12	135,3	-3,0	72,6	63,2	+15,5	94,0
IR13	129,3	-7,3	71,7	47,2	-13,8	87,9
IR14	84,8	-39,2	69,8	52,8	-3,5	82,5
PIR-Control	**125,3**		**83,2**	**62,8**		**90,2**
PIR1	159,2	+27,0	96,7	67,8	+8,0	91,6
PIR2	140,7	+12,3	84,9	55,0	-12,4	86,3
PIR3	119,1	-4,9	97,8	66,2	+5,5	100
PIR4	98,4	-21,4	82,3	46,0	-26,7	85,2
GNTY-Control	**113,9**		**86,7**	**62,3**		**88,5**

Physiological parameters such as mean chlorophyll content, assimilation rate A, stomatal conductance g_s and transpiration rate E under normal and deficit irrigation are shown in Table 2 for all genotypes evaluated. Reduction in water supply was associated with decreased chlorophyll content (SPAD) during the seed filling stage in all genotypes evaluated in both irrigation treatments. In normal irrigation there were no statistically significant differences between the second generation lines and their original genotypes for SPAD1 and SPAD4 measurements. Genotype IR2 showed a statistically significantly lower chlorophyll concentration at the level of P <0,05 (38,2) in the SPAD1 measurement compared to the initial variety Iro (44,2). Also, the lines PIR2 and PIR4 had a statistically significant reduction in SPAD1 under reduced irrigation compared to the original variety Pirgetos. At the beginning of seed filling there were no statistically significant differences in the reduced irrigation between the 2nd generation lines and the original variety Iro. Genotype PIR2 had a significantly reduced chlorophyll concentration (29,7) compared to the original Pirgetos variety (40,1). Chlorophyll content has been proposed as a good indicator of green color and the stay green characteristic under water stress is a commonly observed phenomenon (Fotonat *et al.*, 2007). All the physiological parameters measured differed statistically significant between the two irrigation treatments as shown by the ANOVA analysis (data not shown). Differences in assimilation rate, stomatal conductance and transpiration rate were observed between the 2nd generation lines and their original varieties in both normal and reduced irrigation conditions but did not differ statistically significant (Table 2).

Table 2. Mean chlorophyll content (SPAD1 and 4) during start of anthesis and early seed-filling stage, assimilation rate A (μmol CO_2 m^{-2} s^{-1}), stomatal conductance g_s (mol of H_2O m^{-2} s^{-1}) and transpiration rate E (mol of H_2O m^{-2} s^{-1}) at ultra-low density under normal and deficit irrigation for selected plants of the eighteen second generation lines and the controls evaluated.

	Normal Irrigation					**Deficit Irrigation**				
Second generation lines	**SPAD1**	**SPAD4**	**A**	**g_s**	**E**	**SPAD 1**	**SPAD 4**	**A**	**g_s**	**E**
IR-Control	**41,1**	**37,0**	**15,00**	**0,16**	**2,82**	**44,4**	**36,7**	**16,87**	**0,16**	**2,99**
IR1	41,2	34,8	17,13	0,20	3,38	42,6	31,9	12,64	0,10	2,07
IR2	47,2	44,2	20,96	0,27	4,22	38,2 *	33,1	10,95	0,12	2,15
IR3	40,6	36,3	14,27	0,15	2,89	41,7	34,8	10,32	0,07	1,40
IR4	43,5	34,5	16,78	0,19	2,89	41,4	30,3	10,83	0,10	1,92
IR5	40,1	38,7	13,92	0,13	2,55	44,7	34,5	12,40	0,10	2,22
IR6	43,1	36,6	19,66	0,28	4,00	43,7	31,1	12,45	0,11	2,35
IR7	45,9	34,9	16,64	0,16	2,79	42,7	38,4	15,10	0,15	2,67
IR8	43,0	34,5	16,27	0,27	3,65	43,6	30,4	14,04	0,15	2,73
IR9	37,6	34,7	18,99	0,28	4,27	41,9	32,2	15,46	0,13	2,94
IR10	40,3	31,1	16,22	0,15	2,77	41,8	31,6	13,29	0,12	2,24
IR11	37,7	39,6	18,95	0,24	4,15	43,6	32,1	10,50	0,12	2,28
IR12	41,7	34,0	21,88	0,31	3,89	43,9	31,6	12,80	0,11	2,54
IR13	45,0	33,4	18,27	0,22	3,34	44,7	32,5	8,67	0,05	1,32
IR14	45,3	35,5	17,92	0,23	3,53	42,4	34,3	14,69	0,17	2,75
PIR-Control	**42,8**	**32,8**	**19,67**	**0,33**	**3,85**	**46,2**	**40,1**	**14,38**	**0,14**	**2,50**
PIR1	44,5	30,5	21,67	0,35	4,46	43,1	33,6	10,49	0,09	1,82
PIR2	40,6	38,8	21,00	0,26	4,43	42,2 *	29,7 *	11,16	0,09	2,19

PIR3	43,2	34,0	17,93	0,26	3,71	43,2	30,6	14,14	0,15	2,83
PIR4	42,0	40,0	17,80	0,25	3,59	42,3*	31,2	14,19	0,13	2,16
GNTY-Control	**46,7**	**37,3**	**15,82**	**0,16**	**2,87**	**45,9**	**43,0**	**12,98**	**0,11**	**2,21**

*, Denotes significant difference to the mother landrace (t test for independent means and different standard deviations at the level P<0,05)

CONCLUSION

The results of this study demonstrate that there is intracultivar variation mainly on seed yield under normal and deficit irrigation during anthesis and seed filling stage within second generation sister lines. Compared to the original variety Iro, four of the second generation lines had similar or higher yield plant^{-1} (up to 15%) and exhibited the highest stability under both normal and water deficit conditions, whereas for the variety Pirgetos two progenies outperformed the original cultivar (by 5,5 to 27%). Further research is under way for a final evaluation of the selected progenies under farming density and water stress.

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