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SINGLE-PLANT SELECTION AT ULTRA-LOW DENSITY OF FIRST GENERATION LINES OF THREE BEAN CULTIVARS UNDER WATER STRESS

SUMMARY

Nil-competition (ultra-low plant density) has been asserted to highlight individual genotypes of high yielding potential. This was tested preliminary on three determinate type bean varieties (*Phaseolus vulgaris* L.), two genetically non-uniform and with unstable yields Greek cultivars, Iro and Pirgetos and a "Great northern" type imported variety. Single-plant selection under ultra-low density (interplant distance of 100 cm) was performed in a honeycomb design experiment established during 2017 in the main farm of the University of Western Macedonia in Florina. Eighteen high yielding plants were selected and seed of each constituted a separate first generation line. In 2018, progeny evaluation was conducted in two R21 honeycomb design trials under normal and deficit irrigation treatments respectively. Compared to the original variety Iro, four of the high yielding progeny lines had higher yield plant^{-1} (by 20 to 39%) under water deficit with two being significantly different, where for the variety Pyrgetos only one first generation sister line significantly outperformed the original cultivar by 28%. Water stress affected significantly total chlorophyll content measured at 10 day intervals from start of flowering until physiological maturity with the best performing progeny lines showing higher chlorophyll concentrations especially during the seed filling stage. Significant differences between progeny lines and the original varieties were also shown on CO_2 assimilation rate under water deficit especially within the genotype Iro. Further research is needed so that any existing variation is beneficially exploited.

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

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most important food legume, representing near 50% of grain legumes for human consumption and a significant

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source of high quality and low cost protein (Beede *et al*., 2013). Common bean is the most significant among the other pulses in Greece with increased cultivated areas in recent years (Kargiotidou *et al.*, 2019). 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 methods of classical improvement and main criterion plant yield (Papadopoulos *et al.*, 2007). An intracultivar single-plant selection under ultra-low density has been extensively used to exclude plant-to-plant interference for resources as nil-competition boosts phenotypic expression and erases the masking effects induced by the negative relationship between yielding and competitive ability (Tokatlidis, 2015). This is making selection of desirable genotypes within a narrow gene pool and divergent environments applicable (Vlahostergios *et al*., 2018).

Climate change is inflicting a high impact on agriculture by altering the spatial and temporal distribution of rainfall, which limits water availability (Crimmins et al., 2011). 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*., 2018). Reduced water availability results in lower water potential of plant tissues which decreases stomatal closure, leading to a reduction of CO_2 availability and, consequently, lower photosynthesis and transpiration rates (Teran and Singh, 2002; Bota *et al.*, 2004). 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). However most bean genetic diversity and bean populations are underutilized because of the difficulties that exist with the evaluation of physiological drought response dynamics in many cultivars.

The main objective of the present study was to evaluate high-yielding first generation lines of three different bean varieties selected at ultra-low density under water deficit conditions during anthesis and seed-filling growth stage. The range of variation in agronomic and physiological parameters that could exist may be utilized for identifying and developing improved genotypes which could perform better under adverse conditions.

MATERIAL AND METHODS

Plant material and experimentation

Three common bean determinate type genotypes, two Greek cultivars Iro and Pirgetos developed by the Hellenic Industrial and Fodder Crops Institute and an imported one Great-northern type constituted the source material. During 2017, single-plant selection was performed under ultra-low density (interplant distance of 100 cm) in a honeycomb design experiment in the main farm of the University of Western Macedonia in Florina as described in previous work (Papathanasiou *et al.*, 2018). Eighteen high yielding plants were selected and seed of each constituted a separate first generation line. The selected first generation lines were coded hereafter GNTY1 to GNTY6, IR1 to IR6 and PIR1 to PIR6 according to the original genotype. During the 2018 season, approximately 50 plants per first generation line were assessed in two R21 honeycomb design trials under normal and deficit irrigation treatments respectively using the original genotypes as controls. The experiments were sown on 9th 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 6.3, 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. A drip-irrigation water supply system of 4 L h^{-1} was established along every row, with emitters spaced at 40 cm intervals. Irrigation scheduling was based on bean evapotranspiration (ET_c) and was applied when the crop evapotranspiration rate ET_c - P (rainfall) reached 30 mm. Soil water content at this level was approximately 70% of field capacity, which is considered adequate for plant growth during all stages. The ET_c was calculated from climatic parameters measured daily from a meteorological station located adjacent to the experimental site and was used to calculate the reference evapotranspiration rate (ET_o) using the Penman–Monteith method (Allen *et al.*, 1998). The ET_c, which is the product of ET_o and the crop coefficient (K_c), was calculated using values for bean K_c adjusted to Greek conditions (K_{cini} = 0.35, K_{cropd} = 0.70, K_{cmid} = 1.10, and K_{cend} = 0.30) for growth stages of 15/40/75/95 d after emergence.

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 flowering 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

Mean yield and coefficient of variation (CV%) for seed yield plant^{-1} (g) at ultra-low density under normal and deficit irrigation for the first generation lines compared to the three original genotypes (GNTY, IR and PIR) are presented in Table 1. For the genotype GNTY the six high-yielding first generation lines performed equally with the original genotype under the deficit irrigation treatment with no significant differences in yield plant^{-1} whereas under normal irrigation the control showed higher values than all of its progenies. Compared to the original variety Iro, almost all progeny lines had significantly higher yields under normal irrigation and four of them showed higher yield plant^{-1} (by 20 to 39%) under water deficit with two being significantly different. The first generation sister lines IR1 and IR6 yielded on average 121,2 and 117 g plant^{-1} and showed a CV of 45,5 and 50% respectively. The respective values of the mother genotype IR-control under the same irrigation conditions were 86,8 g plant^{-1} and CV of 55,6%. Similarly for the variety Pirgetos all progeny lines yielded higher than the control under normal water regime but only one first generation sister line significantly outperformed the original cultivar by 28% under the deficit irrigation. The line PIR5 showed mean yield plant^{-1} 120,4 g and CV 52,6% compared to the PIR-control which yielded 94 g with a similar CV of 52,6%. This is in agreement with other studies where under adverse conditions such as high temperatures and increased biotic stress first generation sister lines of bean and/or other legumes such as lentils, outperformed the original genotypes under ultra-low density (Papadopoulos *et al.*, 2004; Vlahostergios *et al.*, 2018). The CV values under the ultra-low density for seed yield plant^{-1} revealed a moderate spatial heterogeneity under deficit irrigation for all the genotypes tested. This is desirable because phenotypic screening and breeding for high yield is expected to ultimately select for potentially tolerant to water stress genotypes (Tokatlidis, 2015).

Physiological parameters such as mean chlorophyll content, assimilation rate A, stomatal conductance to water vapour 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. The high-yielding progenies IR1, IR5 and IR6 had significantly higher values than the IR-control during the late seed-feeling stage (SPAD 5). Similar results were observed for the line PIR5 with significant differences only during the early seed-filling stage (SPAD4). 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).

Table 1. Mean yield and coefficient of variation (CV%) for grain yield plant^{-1} (g) at ultra-low density under normal and deficit irrigation for the first generation lines originating from the three genotypes (GNTY, IR and PIR) and the control.

	Normal Irrigation		**Deficit Irrigation**	
First generation lines	**Yield g plant^{-1}**	**CV%**	**Yield g plant^{-1}**	**CV%**
GNTY1	147,6*	45,2	91,6	55,6
GNTY2	158,3	44,2	82,8	66,7
GNTY3	149,9	55,6	90,0	83,3
GNTY4	152,6	43,3	92,1	62,5
GNTY5	153,4	44,4	91,0	58,8
GNTY6	164,3	44,2	98,4	62,5
GNTY-Control	**176,7**	**37,6**	**90,6**	**58,8**
IR1	161,8**	40,5	121,2**	45,5
IR2	147,3	51,0	100,5	43,5
IR3	158,3*	48,8	112,7	62,5
IR4	152,5*	40,7	82,4	58,8
IR5	177,4**	48,3	104,6	55,6
IR6	178,0**	50,0	117,0*	50,0
IR-Control	**115,4**	**56,5**	**86,8**	**55,6**
PIR1	176,7	34,7	96,0	71,4
PIR2	158,5**	46,3	84,6	58,8
PIR3	153,7*	56,2	81,8	62,5
PIR4	144,0	51,8	91,3	52,6
PIR5	168,0**	44,4	120,4*	52,6
PIR6	141,9	44,2	97,9	55,6
PIR-Control	**117,0**	**50,3**	**94**	**52,6**

*, ** Denotes significant superiority to the mother landrace (t test for independent means and different standard deviations at the levels P<0.05 and P<0.01 accordingly)

Compared to the original variety Iro the first generation sister lines IR1 and IR6 showed significantly lower reduction in A, g_s and E. The higher stomatal conductance of these two progenies under the water deficit conditions led to an increased CO_2 availability which had a direct positive effect on photosynthesis compared to the IR-control. Similar results have been reported by Soureshjani *et al.* (2018). Although the higher yielded progeny PIR5 had a better physiological response than the PIR-control no significant differences were observed.

Table 2. Mean chlorophyll content (SPAD 4 and 5) during early and late seed-filling stage at two intervals of 10 days, assimilation rate A (□mol CO_2 m^{-2} s^{-1}), stomatal conductance to water vapour 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 the first generation lines originating from the three genotypes (GNTY, IR and PIR) and the control.

	Normal Irrigation					**Deficit Irrigation**				
First generatio lines	**SPAD 4**	**SPAD 5**	**A**	**g_s**	**E**	**SPAD4**	**SPAD5**	**A**	**g_s**	**E**
GNTY1	39,22*	32,98*	13,78	0,360*	3,91	35,60	28,22	9,20	0,200	2,60
GNTY2	45,67	37,57	15,74	0,592	4,84	33,52*	25,35*	8,33	0,123*	2,16*
GNTY3	41,82	33,97*	14,92	0,482	4,35	35,87	26,92	7,47	0,138	2,26*
GNTY4	43,73	38,92	14,33	0,488	4,11	41,53	30,82	8,91	0,125*	2,38*
GNTY5	40,23*	34,47	16,85	0,513	5,62	38,98	23,93*	8,35	0,126*	2,19*
GNTY6	40,98*	31,43	14,22	0,460	5,04	39,35	26,78*	9,75	0,193	2,45
GNTY-Control	**44,68**	**37,40**	**16,07**	**0,462**	**4,92**	**39,48**	**31,70**	**10,49**	**0,218**	**3,34**
IR1	39,32	34,50	16,20*	0,432	4,76	38,85	31,58*	12,56*	0,230*	2,53
IR2	41,37	33,88	11,84	0,410	3,85	35,68	29,63	8,92	0,147	2,34
IR3	40,10	32,87	14,22	0,443	4,31	38,03	28,33	12,36*	0,188	2,81
IR4	43,12*	40,07	14,10	0,478	3,94	32,83	25,52	7,85	0,130	2,35
IR5	43,75*	37,50	19,36*	0,497	5,14	43,88*	30,93*	12,01	0,208	2,76
IR6	42,02	37,10	18,18*	0,525*	5,31	40,45	31,43*	12,81*	0,237*	3,29*
IR-Control	**39,15**	**32,43**	**11,59**	**0,390**	**4,36**	**36,87**	**26,02**	**8,90**	**0,138**	**2,33**
PIR1	45,10*	38,83*	18,99*	0,502	5,16	34,75	28,55	10,70	0,153	2,41
PIR2	42,57*	36,27*	13,88	0,478	4,14	36,47	29,18	8,18	0,230	3,10
PIR3	41,92*	34,63	15,22	0,480	4,27	32,15*	27,08	8,69	0,182	2,49
PIR4	40,70*	34,37	13,57	0,488	4,00	37,45	31,32	10,17	0,203	2,84
PIR5	44,18*	36,25*	15,10	0,392	4,26	42,82*	30,13	11,88	0,213	2,74
PIR6	40,70*	32,28	13,85	0,475	4,81	40,95	28,63	11,30	0,173	2,91
PIR-Control	**37,12**	**32,05**	**13,96**	**0,600**	**4,57**	**37,58**	**28,43**	**11,02**	**0,155**	**2,58**

*, ** Denotes significant superiority to the mother landrace (t test for independent means and different standard deviations at the levels $P<0.05$ and $P<0.01$ accordingly)

CONCLUSIONS

The results of this study demonstrate that there is intracultivar variation on seed yield under deficit irrigation during a thesis and seed filling stage within first generation sister lines. Also physiological traits were related to deficit irrigation tolerance which could assist in the identification of mechanisms underlying these adaptation processes and in the selection of improved genotypes of common bean. Further research is underway to confirm the results of the present study and to exploit further any existing variation.

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