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Seed Germination and Seedling Growth of Three Lentil Cultivars under Moisture Stress

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Abstract: The aim of this study was (I) to investigate the response of three lentil cultivars [FLIP 86-16 L (large seeds), FLIP 89-31 L (small seeds) and FLIP 95-3L (small seeds] to osmotic stress (-0.33, -0.66, -0.99 and -1.72 Mpa) during germination and seedling growth, (ii) to identify characters that can be used for screening genotypes, (iii) to determine the effects of cultivars on yield and yield components of rainfed lentil in arid (150 mm rainfall) and semiarid (364 mm) regions. Large seed cultivars had higher percentage germination and germination speed under moisture stress than small seed cultivars, but germination speed was more sensitive to change the osmotic potential than percentage germination. Root and shoot weights of all cultivars were reduced when osmotic potential was decreased, but the extent of reduction in root growth was less than that for shoots. Lentil plants at the semi-arid location (Houfa) had higher seed yield (kg ha^{-1}), 1000 seed weight (g) and plant height (cm), than those grown at the arid location (JUST). Lentil plants from larger seeds cultivar had higher seed yield, 1000 seed weight and plant height than those from smaller seeds cultivars.

Key words: Seed germination, seedling growth, lentil, moisture stress

INTRODUCTION

Lentil (*Lenus culinaris* Medic) is a major grain legume crop in many developing countries in West Asia and North Africa and many other areas of the world. In Jordan lentil is mainly consumed as soup or "Mjddara" , both of which serve as a popular dish and are rich in a relatively cheap source of protein. This crop is cultivated under rainfed conditions in these countries. These countries are characterized by Mediterranean climate. One of the major problems facing rainfed agriculture of these regions is drought which sometimes restricts emergence and early seedling growth[1], which may subsequently affect winter survival of plants. Using factors which enable plants to better adapted to prevailing environmental conditions could help improve crop production. Moisture stress effects every aspect of plant growth and metabolism. The germination phase is of prime importance in the growth cycle of plants as it determines the stand establishment and final yield of the crop[2]. Water stress may result in delayed and reduced seed germination or may prevent germination completely. Taylor *et al.*[3], demonstrated that seed germination and emergence are reduced at a smaller negative osmotic potential although the exact potential inhibiting germination varied considerably. Moreover, it has been found that initiation of cell elongation and cell elongation itself during germination are differentially sensitive to water stress[4].

The aim of this study was (I) to investigate the response of differentially drought tolerant lentil cultivars to osmotic stress during germination and seedling growth (ii) to identify seed and seedling traits that can be used in screening genotypes for drought tolerance, (iii) to determine the effects of cultivars on yield and yield components of rainfed lentil in arid (150 mm rainfall) and semiarid (364 mm) regions.

MATERIALS AND METHODS

Laboratory experiments: Three lentil cultivars [FLIP 86-16L (large seed size, 0.9 cm), FLIP 89-31 L and FLIP 95-3L (small seeds cultivars, 0.4 and 0.5 cm, respectively) were used in this study. Degree of drought tolerance was mainly based on yield comparisons under varying levels of water stress.

Seed germination: Seeds of the three lentil cultivars were germinated in covered petri dishes between filter paper moistened with solutions of five osmotic potentials. Three layers of Whatman No. 2 filter paper disks were used in each dish, two below the seeds and one on top. Six ml of

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distilled water (0 Mpa) or polyethylene glycol 8000 solutions of –0.33, -0.66, -0.99 and –1.72 Mpa were added to petri dishes, each containing 25 seeds treated with Captan 50 (N-[trichloro-methylthio]-cyclothexene- 1,2-dicarboximide). Petri dishes were then placed in a dark germinator maintained at a constant temperature of 15°C. The concentration of PEG-8000 needed to produce each osmotic potential was obtained from the formula development by Michel and Kaufmann[5]:

$$\Psi s = -(1.18 \times 10^{-2})\ C - (1.18 \times 10^{-4})\ C^2 + (2.67 \times 10^{-4})\ CT + (8.39 \times 10^{-7})\ C^2T$$

Where, C is concentration of PEG-8000 in g L^{-1} of water, T is temperature in °*C* and Ø¾ is osmotic potential in bars (converted to Mpa by dividing by 10). Each experimental unit consisted of two petri dishes, or 50 seeds, replicated four times in a completely randomized design in a factorial experiments, with osmotic potential and cultivars as the factors. Germination counts that were taken every 24 h for 21 days. Seeds with a radical extension equal to greater than 2 mm were considered as germinated, counted and then discarded. Seeds that developed fungal growth were removed from the dishes and considered as dead. Percentage germination was calculated based on the total number of germinated seeds per treatment after eight days of incubation. The speed of germination index (SGI) was calculated according to the following formula:

$$SGI = {}^{c}\Sigma I = iNi/ Ti$$

Where, Ti is the germination cont day, Ni is the number of seeds germinated on day T and C is the number of days counts that were made in the test (21 days). Percentage germination values were transformed before analysis by arcsine square root of percent to normalize the variance[6].

Seedling development: This experiment consisted of five osmotic potential treatments replicated eight times in a split plot arrangement of a completely randomized design, with osmotic as main plots and the five cultivars as subplots. Randomly chosen five-day old seedlings from each cultivar were placed on perforated styrofoam sheets (0.5 cm thick) floating on aerated half strength Hoagland solutions[7], having osmotic potentials of –0.33, -0.66, -0.99 and –1.72 Mpa, plus control treatment with osmotic potential close to 0 Mpa. Seminal roots were inserted through the perforations and were the only part of the seedling in contact with the solution. Each styrofoam sheet had a total of 100 seedlings, 20 from each cultivar, floating on 500 ml of solution. Seedling were then allowed to grow for 15 days in a growth chamber at 15°C and 90 % RH, with altering light-dark periods of 16 and 8 h, respectively. Data was collected on fresh and dry weight of shoots and root. Shoot and root fresh weights were determined after separating the roots from the shoots at the seed juncture and discarding the seed and dry weights determined using dry weight values.

Field experiments: To determine the effects of cultivars on yield and yield components of rainfed lentil in arid (150 mm rainfall) and semiarid (364 mm) regions, the present study was conducted at two sites in northern Jordan, which has a Mediterranean climate: mild and rainy in winter; dry and hot in summer. One was a field site of the Jordan University of Science and Technology (JUST) campus (an arid region which received 150 mm of rainfall). The other site was situated in the semi arid village of Houfa, which received 364 mm of rainfall.

At the two locations, the three lentil cultivars [FLIP 86-16 L (large seed size), FLIP 89-31 L and FLIP 95-3L (small seeds cultivars) were grown in a randomized complete block design (RCBD) with four replications. Plots were 2.5 m long and 2.4 m width. Each plot contained 6 rows, spacing between and within rows was 0.4 and 0.1 m, respectively, resulted in planting density of about 25 plants m^{-2}. Guard strips of 1.5 m between the plots and 2 m between replications were left non- planted. Fertilizer was hand broadcasted prior seeding at a rate of 20 kg ha^{-1} of nitrogen and 40 kg ha^{-1} of P_2O_5. Weeds were removed manually as needed.

Measured variables seed yield (kg ha^{-1}), 1000 seed weight (g) and plant height (cm). The analysis of variance and LSD mean separation were performed using computer statistical program MSTAT-C as described by Steel and Torrie[6]. Comparisons between means were made using the least significant difference test (LSD) at 0.05 probability level.

RESULTS AND DISCUSSION

Laboratory and growth chamber experiments

Seed germination: Osmotic potential and cultivar had a significant effect on germination results Lowering the osmotic potential from 0 to -0.33 Mpa led to slight, significant reductions in germination of all cultivars except FLIP 86-16 L, a drought tolerance cultivar (Table 1). Similar results were reported earlier in lentil cultivars[1] who, indicated the declines in germination percentage of lentil seeds with increasing level of water stress. The reduction in germination percentage may be attributed to lower diffusiblity of water seed coats at greater water stresses.

Table 1: Percentage germination and speed of germination index of three lentil cultivars imbibed in solutions of different osmotic potentials

Cultivar	Osmotic potential Mpa	Percentage germination	Seed index germination
FLIP 86-16 L	0	98	23
	-0.33	97	18
	-0.66	95	17
	-0.99	82	15
	-1.72	60	2.0
FLIP 89-31 L	0	92	18
	-0.33	88	15
	-0.66	80	12
	-0.99	60	10
	-1.72	35	1.5
FLIP 95-3L	0	93	19
	-0.33	90	16
	-0.66	83	13
	-0.99	63	11
	-1.72	40	2.0
LSD (0.05)		2.1	1.3

Table 2: Seedling shoot and root fresh and dry weights of three lentil cultivars imbibed in solutions of different osmotic potentials

		Fresh weight (mg plant^{-1})		Dry weight (mg plant^{-1})	
Cultivar	Osmotic potential Mpa	Shoot	Root	Shoot	Root
FLIP 86-16 L	0	230	120	18.9	2.4
	-0.33	199	97	14.0	2.0
	-0.66	80	70	6.3	1.8
	-0.99	35	40	2.4	1.6
	-1.72	25	35	2.4	1.4
FLIP 89-31 L	0	200	111	16.0	2.1
	-0.33	100	90	13.0	1.8
	-0.66	20	35	4.7	1.3
	-0.99	15	25	2.2	1.1
	-1.72	7	17	1.9	1.1
FLIP 95-3L	0	205	115	16.6	2.1
	-0.33	97	90	11.3	1.7
	-0.66	22	33	3.7	1.4
	-0.99	13	21	1.8	1.2
	-1.72	8	15	1.4	1.2
LSD (0.05)		12.0	14.3	0.7	0.6

Table 3: Seed yield (kg ha^{-1}), 100 seed weight (g) and plant height (cm) of three lentil cultivars grown under arid (JUST) and semi arid(Houfa) locations

Cultivar	Seed yield (Kg ha^{-1})	1000 seed weight (g)	Plant height (cm)
FLIP 86-16 L	650.0	67.5	47.5
FLIP 89-31 L	500.0	45.0	30.0
FLIP 95-3L	535.0	48.5	33.0
LSD (0.05)	37.0	4.5	6.3
Locations			
Semi-arid (Houfa)	447.0	59.3	25.6
Arid (JUST)	677.0	48.0	48.0
LSD (0.05)	59.0	5.2	5.3
Interaction	NS	NS	NS

Initial water uptake of leguminous seeds decreased as external water potential decreased[2]. At on osmotic potential of -0.66 Mpa, a significant drop in percentage germination was observed for small size cultivars FLIP 89-31 L and FLIP 95-3L , while the large size cultivars, FLIP 86-16 L, exhibited no such reduction. However not of the cultivars, had percentage germination lower than 80% at that osmotic potential (Table 1). The critical osmotic potential, leading to substainl reductions in germination for all cultivars, was between -0.66 and -1.72 Mpa, when the osmotic potential was reduced from -0.66 to -1.72 Mpa germination of FLIP 89-31 L and FLIP 95-3L decreased by 56.3 and 51.8% respectively. However, the large size cultivars FLIP 86-16 L, still had significant higher percentage germination than FLIP 89-31 L and FLIP 95-3L which exhibited reductions in germination. Similar to percentage germination, seed of germination was also affected by cultivar and osmotic potential. However, unlike germination, the speed of germination of all cultivars was consistently and significantly reduced whenever the osmotic potential was reduced, up to -1.72 Mpa (Table 1). Most of the differences between cultivars were observed at -0.33 Mpa, where the large size cultivars FLIP 86-16 L had significantly higher speed of germination than FLIP 89-31 L and FLIP 95-3L the small seed cultivars. These results are inconsistent with Mian and Nafziger[8] who reported that seed size had no effect on germination percentage in wheat (*Triticum aestivum*).

Seedling development: Reducing the osmotic potential from 0 to -0.33 Mpa led to significant reductions in shoot fresh weight of all cultivars, with FLIP 95-3L, the small seed cultivar, showing the greatest reduction in fresh weight and the large seed cultivar FLIP 86-16 L the least reduction, compared to their respective controls (Table 2). This was confirmed by several published results on different crops[8]. Plants from larger seeds had grater rates of stem elongation, higher leaf area and higher shoot, leaf and root dry weights than those from smaller seeds of common vetch (*Vicia sativa* L.)[9]. This positive relationship was associated with the positive correlation between seed weight and cotyledonary reserves found in leguminous seeds[10]. In general, plants produced from large seed grew faster, accumulated more above ground biomass and produced higher grain yields than plants from small seeds[11]. Longer roots in large compared to small seeds were noted at –0.33, -0.66 and -0.99 Mpa water potential levels. These results might indicate that large lentil seeds have beneficial effects through a rapid penetrating root system than small seeds, at relatively intermediate water potentials. This is important for lentil grown in semi arid areas (intermediate soil moisture conditions), in order to extract more moisture from deep soil layers. These results are consistent with those of Mian and Nafziger[8] when wheat plants had higher root growth under high water potentials (less stress) than under low water potential (more stress).

Root fresh weight of all cultivars was significantly reduced when the osmotic potential was lowered to -0.33

Mpa, with FLIP 95-3L exhibiting the greatest reduction relative to the control (Table 2). Reducing the osmotic potential further to -0.99 Mpa led to further significant reductions in fresh weight of all cultivars, with small seed cultivars showing a higher degree of reduction than large seed cultivars compared to their respective controls.

The greatest reduction in shoot dry weight was observed when the osmotic potential was lowered from -0.33 to -1.72 (Table 2). Dry weight of all cultivars significantly decreased whenever the osmotic potential was decreased up to -1.72 Mpa and that FLIP 95-3L showed a further significant decrease at -1.72 Mpa. Although significantly affected by osmotic potential, the extent of reduction in root dry weight with decreasing osmotic potential was less than observed for shoot dry weight. FLIP 86-16 L as drought tolerant based on yield under limited soil moisture, had higher percentage germination under moisture stress than either FLIP 89-31 L or FLIP 95-3 L, both rated as drought susceptible (Table 1).

Field experiment: Statistical analysis showed no significant interaction for seed yield, 1000 seed weight and plant height between the cultivars and locations.

Lentil plants at the semi-arid location (Houfa) had higher seed yield (kg ha^{-1}), 1000 seed weight (g) and plant height (cm), than those grown at the arid location (JUST) (Table 3). The higher seed yield may have been because of favorable moisture as well as to the longer life cycle of plants at Houfa than at JUST. Moreover, the plants at JUST flowered and reached to physiological maturity earlier than those at Houfa. Many breeding strategies for yield increases are based on the assumption that increased yield potential depends on increases in size of the photosynthetic source, achieved through lengthening the growth cycle[12]. Increases in crop yield arise from the amount of solar radiation plants intercept to lengthen the life of the canopy. Lengthening life of plant canopies has been associated with increased yields[13]. Overal locations plants from larger seeds cultivar (FLIP 86-16 L) had higher seed yield, 1000 seed weight and plant height than those from smaller seeds cultivars (FLIP 89-31 L and FLIP 95-3L).

In conclusion, water stress induced by imposing soil water deficits was determined to effect both germination and plant growth of lentil . Seed size had significant effect on germination under the range of water stresses impose in those experiments. Even though the effects of seed size on lentil seed yield may depend largely on environmental growing conditions, the present study indicated that larger seeds produced larger plants and this phenomenon was relatively more pronounced under intermediate water stress than under well-watered or more severe water stress conditions.

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