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RESEARCH ARTICLE (Open Access)

Early selection of kabuli chickpea genotypes (*Cicer arietinum* L.) tolerant to osmotic water stress

KAMEL BEN MBAREK[1*] MOHSEN BOUBAKER[2]

[1]Department of Horticultural Systems Engeneering, High Agronomic Institute of Chott-Mariem, IRESA-University of Sousse, Tunisia

2Department of Horticulture, High Agronomic Institute of Chott-Mariem, IRESA-University of Sousse, Tunisia

Abstract

Eight " kabuli " chickpea genotypes $Beja_1$, $Amdoun_1$, Nayer, Kasseb, Bochra, FLP96-114C, FLP88-42C and Chetoui were germinated, in two *in vitro* culture media, particularly, agar and filter paper Watman n°2 and under tree osmotic water pressures (OWP): -0,33; -4 and -8 bars induced by PEG_{8000}. On filter paper, germination appeared more accelerated with a higher rate compared to the agar media. Osmotic water stress has negatively affected the seeds germination and the seedlings vegetative development parameters. Osmotic water pressure - 8 bars completely inhibited seeds germination on filter paper media. On the other hand, on agar media, it caused a feeble germination rate and a stunting of the seedlings. A broad genotypic variability of the chickpea cultivars was revealed toward the osmotic water stress. Tolerance index to osmotic water stress revealed three groups of cultivars: (1) Nayer and Kasseb are tolerant, (2) Bochra, FLIP88-42C and Chetoui are fairly tolerant and (3) $Amdoun1_,$ $Beja_1$ and FLIP96-114C are sensitive to this abiotic stress.

Key words Chickpea; Osmotic water stress; PEG_{8000}; Culture media.

1. Introduction

Around the world, drought and salinity are the most important abiotic stresses that limit production [31]. Toker, [35] reported, in North Africa, drought is the most important constraint of chickpea (*C. arietinum* L.) production. In the Mediterranean area, this species is commonly planted in the spring and may suffer from dry, especially, in case of delayed sowing [36]. Processes related to plants growth and development is affected by water deficit [39]. Boubaker and Yamada [6] found that germination in dry soil produces plants of low vigor. Under water deficit conditions, the ability of seeds to produce vigorous plants indicates that they have potential tolerance to water stress. Blum and Ebercon [4] indicated that under drought conditions, seed germination and plant growth are rapid and reliable criteria for screening drought tolerant genotypes. Drought has a physiological action which results in the creation of osmotic water stress in the plant [31]. Polyethylene glycol (PEG), a non-ionic hydropolymer that does not rapidly enter in plant tissues, is widely used to induce osmotic water stress [31]. Sané, *et al.*, [31] found that, *in vitro* culture, the use of PEG allows quick and easy identification of water stress tolerant genotypes. This technique has been commonly used to assess the level of drought tolerance of wheat cultivars. Tolerance index to osmotic water stress allows a direct comparison of genotypic responses to osmotic water stress [6].

2. Materials and methods

2.1 Plant material

Eight Kabuli chickpea genotypes six out of which were from Tunisia: $Beja_1$, $Amdoun_1$, Nayer, Kasseb Bochra and Chetoui and the last two: FLIP88-42C and FLIP96-114C provided by ICARDA (International Centre of Agricultural Research Dry Areas) Aleppo, Syria, were grown *in vitro* culture with the aim to evaluate their tolerance to osmotic water stress induced by PEG_{8000}.

2.2 Cultivation

Chickpea seeds were sterilized in 6 % sodium hypochlorite for 5 min and 75 % ethanol for 3 min. Subsequently, they were rinsed with distilled water. Germination was tested on culture media containing 7 g/L agar in 500 ml bottles, and between two layers of Whatman filter paper N° 2 on Petri dishes [12]. Three OWP: -0.33, -4 and -8 bars, were induced by the addition of the respective doses of PEG_{8000}: 0; 16,75 and 23 g/100 ml in the agar media or 100 ml of

Correspondence: Kamel Ben Mbarek; Department of Horticultural Systems Engeneering, High Agronomic Institute of Chott-Mariem, IRESA-University of Sousse, Email: benmbarekkamel@yahoo.fr
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distilled water of filter paper. Ten seeds per genotype were sowed in each bottle and Petri dishes following a split block experimental design with three replications. Germination was conducted 22 °C temperature, 70 to 80% relative humidity, 2500 lux luminous intensity and 10/14h daily photoperiod [12]. Seeds were considered germinated when the radicles pierced the seed coat or were clearly elongated [8].

2.3 Recorded parameters

Germination parameters recorded were:

- Germination rate (GR, %): The number of germinated seeds by the total number of seeds placed in germination in bottle or petri dish;
- Germination time or germination speed (GT; days) indicates the number of days to have seed germination. It has been defined by Dirik, [12] using the formula:

$$GT = \sum (n_i \times t_i) / N$$

With: n_i: germinated seeds number in t_i, t_i = number of days after sowing, N: total number of germinated seeds.

- Germinative energy or germinative value (GE: seedlings /day) [13] is the inverse of germination time and indicates the number of sprouts per day.

The vegetative development parameters were defined on the seedlings developed on agar media:

- Radicle length (RL; mm) and Epicotyle length (EpL; mm);
- Rootlets number of which the length was greater than 2 mm (RNb; mm);
- Ratio of the Radicle by Epicotyle Lengths (RL/ EpL);
- Rootlets and Epicotyle Water Content (RWC; %) (EpWC; %) using the formula:

$$WC = 100 * (WW - DW) / DW$$

With WC: water content, WW: fresh weight of rootlets or epicotyle, DW: dry weight of rootlets or epicotyle

Rootlets and epicotyle dry matter Content (RDMC; %) (EpDMC; %) expressed by the formula:

$$DMC = 100 * (DW / WW)$$

With DW: dry weight and WW: fresh weight.

- Ratio of the dry matter rates: RDMC/EpDMC;
- Rootlets fineness (RF; $mm.g^{-1}$ of dry matter) expresses the ratio of the radicle length by the dry weight [41].
- Tolerance index to osmotic water stress as defined by Fischer *et al.,* [16] using the formula:

$$TIOWS = \frac{Parameter…under…water...stressed...conditions}{Parameter…under...non...stressd...water...conditions}$$

The parameter under water stress conditions was the treatment under OWP -8 bars, whereas the parameter under not stressed water conditions was the treatment without PEG_{8000},

Tolerance index to osmotic water stress of each genotype corresponded to the average stress indexes of all parameters that showed genotypic variability. Low indexes indicate sensitivity, whereas high indexes indicate tolerance.

Results were processed by statistical analyzes, including ANOVA, means comparison tests by the Student-Newman-Keuls test (SNK test, P ≤ 0.5%) and the tolerance index to osmotic water stress.

3. Results and Discussion

3.1 Germination parameters

Culture media effect

Variance analysis showed that culture media had highly significant effects (P ≤ 1 ‰) on the germination energy, but were not significant on the germination rate and the germination time of the chickpea seeds (Table 1). Variation coefficients were 20,7 % for the germination energy, 23,2 % for the germination rate and 31.8 % for the germination time. Germination rate and germination time reached similar values on the filter paper and agar ranging from 64 to 66,8 % for the first parameter and from 4,28 to 5,87 days for the second. In contrast, germination energy appears higher on filter paper (1,54 seeds/day) than on agar media (0,96 seeds/day). It seems that the agar has lower water potential than that of the filter paper. Bewley and Black [3] reported that germination is a complex phenomenon that involves many physiological and biochemical changes that lead to activation of the embryo. However, the water deficit caused by the agar low osmotic water potential inhibited seeds rehydration and reduced germination energy.

Pressure level effect

Osmotic water pressure levels had highly significant effects (P ≤ 1 ‰) on the germination rate, germination energy and germination time (Table1). Germination rate was inversely proportional to the OWP and varied from 21,3 to 91,48 %. Mean comparisons (SNK test, P ≤ 0.5 %) showed that the OWP -0.33 bars caused the highest germination rate and OWP -8 bars which generated the lowest one. OWP -4 bars presented a medium germination rate (Table 2).

Germination time varied from 4,13 to 8,37 days. Mean comparisons (SNK test, P ≤ 0.5 %) showed two

distinct homogeneous groups: OWP -0.33 and -4 bars which caused accelerated germination and OWP -8 bars which caused slow germination (Table 2). These results are consistent with those of Jaouadi *et al.,* [18] who observed that the germination time is proportional to the intensity of osmotic water stress. Also, Dirik [12] found that the germination time was slightly higher in stressed treatments compared to unstressed seeds. Germination energy varied from 0,178 to 1,793 seeds /day, being the highest under -0,33 bars and the lowest under -8 bars (Table 2). Turner [39] reported that the germination capacity or germination energy was inversely proportional to the PEG_{8000} culture media concentration.

Table 1. Mean squares and F test of the germination rate and the germinative energy of the chickpea genotypes (*Cicer arietinum* L).

Variation source	Germination rate (%)	Germination energy (seeds/d)	Germination time (d)
CM	280,56ns	12,25***	7,116ns
OWP	71003,13***	41,56***	66,082***
G	2009,67***	0,638***	9,038***
Bloc	1146,382**	0,143ns	2,365ns
CM * OWP	14190,77***	9,62***	1,922ns
CM * G	1900,1***	0,578***	0,769ns
OWP *G	404,77ns	0,228***	9,046***
CM*OWP*G	592,88***	0,228***	1,641ns
Error	231,24	0,067	2,772
Variation coefficient (%)	23,2	20,7	31,8

Ns: not significant, *: significant at 5 % level, ***: significant at 1 ‰ level, CM: culture media, OWP: Osmotic Water Pressure, G: Genotype, d: day.

Table 2. Mean comparisons of the germination parameters of the chickpea genotypes (*Cicer arietinum* L.) according to the osmotic water pressures and the genotypes.

Parameters		Germination rate (%)	Germination time (days)	Germination energy (seeds/day)
OWP (bars)	-0,33	**91,48a**	**4,13a**	1,787a
	-4	83,54b	4,93a	**1,793a**
	-8	**21,25c**	**8,37b**	**0,178b**
Genotypes	$Beja_1$	68,3bc	**6,14a**	1,211ab
	$Amdoun_1$	57,9ab	4,47a	1,144ab
	Nayer	**78,9c**	4,72a	**1,52c**
	Kasseb	67,7bc	5,42a	1,304bc
	Bochra	72,9c	**4,28a**	1,473c
	FLIP96-114C	52,5a	4,29a	1,056a
	FLIP88-42C	74,7c	5,34a	1,32bc
	Chetoui	**50,6a**	**6,14a**	**0,991a**

Numbers of the same column accompanied by the same letter are not significantly different (test SNK, P=0,5 %); Numbers in fat are the extreme average values;

Genotype effect

A highly significant (P ≤ 1 ‰) genotypic variability was detected for the germination rate; germination energy and germination time (Table 1). In fact, Germination rate varied depending on chickpea genotypes from 50,6 to 78,9 %. Genotypes: $Beja_1$, Nayer, Kasseb, Bochra and FLIP88-42C represented a first group with high germination rates. Genotype Nayer had a fairly high germination rate. The last group FLIP96-114C and Chetoui had the lowest germination rates (Table 2). Germination time varied from 4,28 to 6,14 days. Genotypes $Beja_1$ and Chetoui presented the longer germination time; on the contrary Bochra showed the faster germination (Table 2). Germination energy ranged between 0,991 and 1,52 seeds/day. Mean comparisons revealed three interfered genotype groups. The first one ($Beja_1$, $Amdoun_1$, FLIP96-114C and Chetoui) showed low germination energy. The second group formed by genotypes Nayer, Kasseb, Bochra and FLIP88-42C exhibited high germination energy. The last group, composed of $Beja_1$, $Amdoun_1$, Kasseb and FLIP88-42C, showed moderately high germination energy (Table 2). Differences in cultivar response to osmotic water stress can be attributed to differences in structural or physiological traits such as osmoregulation capacity and cell membranes integrity [6],

Interactions between factors

The interactions (CM x OWP) and (CM x G) showed very highly significant (P ≤ 1 ‰) differences

at the germination rate and germination energy and not significant differences at the germination time (Table 1).

We note that, whatever the OWP, germination rates and germination energies were higher on filter paper than on agar. These results indicate that the agar has lower water potential compared with filter paper. It slowed down the germination speed and reduced the chickpea genotypes germination rate and energy. On filter paper and under the pressure -8 bars, the reduced germination rate and germination energy to zero indicates that germination was severely affected by the PEG_{8000} [20]. This could be explained by the fact that PEG_{8000} acts like an osmotic agent by dehydrating seeds [1]. Thus hydrolysis of the seed nutritive reserves and germination were inhibited [12].

Culture media have differently affected chickpea genotypes germination rate and germination energy. Genotypes $Beja_1$, Nayer, Bochra and FLIP88-42C presented higher germination rate on agar compared with filter paper. Inversely, FLIP96-114C and Chetoui showed higher germination rate on filter paper than on agar. Genotypes $Amdoun_1$ and Kasseb exhibited similar germination rates on both culture media. All chickpea genotypes showed higher germination energy on filter paper than on agar.

The interaction (G x OWP) showed very highly significant differences ($P \leq 1$ ‰) for germination energy and the germination time and not significant differences for germination rate (Table 1). These results prove that chickpea cultivars differ in their sensitivity to osmotic water stress and germination energy and germination time can be used as selection criteria for tolerance to osmotic water stress [6].

The lowest germination energy was expressed by the set of genotypes under the pressure -8 bars. Under the pressures -0.33 and -4 bars, genotypes Kasseb, FLIP96-114C and FLIP88-42C showed higher and similar germination energy. Genotypes $Beja_1$, $Amdoun_1$ and Chetoui presented higher germination energy under -0.33 bars than under -4 bars. Conversely, Nayer and Bochra germination energies are higher in -4 bars than in -0.33 bars.

Germination time of the genotypes $Amdoun_1$, Bochra and FLIP88-42C are not affected by the different OWP. On the other hand, the OWP -8 bars have strongly delayed the germination of the genotypes: $Beja_1$, Nayer, Kasseb, FLIP96-114C, FLIP88-42C and Chetoui.

The interaction (CM x OWP x G) showed very highly significant differences ($P \leq 1$ ‰) for germination rate and germination energy, but were not significantly different for the germination time (Table 1). Germination rate and germination energy were negatively affected by the agar culture media and the OWP. Under the pressure -8 bars, on filter paper, the germination of all chickpea genotypes is completely inhibited, whereas on agar, genotypes $Beja_1$, Nayer, Kasseb and FLIP88-42C recorded higher than 60 % germination rates. Under the pressures -0.33 and -4 bars, on both culture media, germination rates recorded by $Beja_1$, Nayer, Bochra and FLIP88-42C were high and similar. Under low OWP -0,33 and -4 bars, on filter paper, germination energy presented by the genotypes Nayer, Kasseb, Bochra, FLIP96-114C and Chetoui was similar and significantly higher than those recorded on agar media.

3.2 Vegetative growth parameters

Culture media effect

Variance analysis showed that OWP had highly significant effects ($P \leq 1$ ‰) on rootlets number per seedling, epicotyle length and elongation speed, seedlings fresh weight, rootlets and epicotyle water content and dry matter content and rootlets fineness. It also had significant effects ($P \leq 5$ %) on radicle length (Table 3). Rootlets number per seedling varied from 0,79 to 3,04 and was inversely proportional to the OWP. It was the highest without PEG_{8000} and the lowest at -8 bars (Table 4). Drought resistance mechanisms are very complex and may involve morphological, physiological and biochemical factors [20]. However, researches showed that water deficit, limited in time, can maintain or even increase, temporarily, speed root elongation and induce the appearance of new short lateral roots [28].

Radicle length per seedling ranged from 19,7 to 39,9 mm. Under low osmotic water pressures (-0.33 and -4 bars) the radicle was relatively long. OWP -4 and -8 bars led to limited development radicle (Table 4). These results are consistent with those of Romo *et al.,* [29]. According to Brown *et al.,* [7], under water deficit conditions, longer root system capable of pumping deep water appears good morphological character. The length and density of roots seems to be good indicators of morphological adaptation to water deficit.

Epicotyle length of the chickpea seedlings was inversely proportional to OWP and ranges from 7,01 to 23,1 mm. Under low OWP -0.33 and -4 bars, seedlings produced long epicotyles with similar values, respectively, 20,3 and 23,1 mm. However, under pressure -8 bars seedlings epicotyles were stunted

(7,01 mm) (Table 4). Our results were similar to those reported by Muhammad and Iram [25].

Epicotyle elongation speed was inversely proportional to the OWP and varies from 0,31 to 1,39 mm/day. The absence of PEG_{8000} accelerated elongation, evaluated at 1,39 mm/day. Lower elongation speed was recorded under the OWP -4 bars. The most attenuated growth speed (0,31 mm/day) was recorded under -8 bars (Table 4). Thus, chickpea seedlings respond to water stress induced by PEG_{8000} by the epicotyle growth inhibition [26].

Under different OWP, the ratio RL/EpL varied from 1,62 to 1,97. Mean comparisons showed a single homogeneous group (Table 4). Turner [39] indicated that *in vitro* culture, this ratio increases with higher PEG concentrations. Sharp, *et al.*, [33] found that the low water potential of the culture media resulted in a reduction of the air biomass without inhibiting the root mass growth and caused an increase in the ratio: root mass / biomass air. Whatever induced OWP, the radicle showed a higher growth compared with epicotyle. It seems that the epicotyle is more sensitive to the PEG_{8000} stress effect than the root system. This result can be explained by the fact that the increase in the OWP culture media blocked the water absorption by the root system and resulted in a decrease in the growth of the vegetative component (Table 4).

Seedlings fresh weight varied from 0,61 g to 1,28 g. Pressures of -0.33 and -4 bars had similar effects on seedlings development, while OWP -8 bars seemed to hinder the seedlings development (Table 4). These results are confirmed those of Mar *et al.*, [22]. This reduction is more important than increasing the PEG concentration in the culture media. Seed ability to produce vigorous plants under water stress conditions indicates a tolerance for this abiotic stress [6].

Water contents in the radicles and epicotyle are inversely proportional to the OWP and vary, respectively, from 661 to 1195 % and from 666 to 1172 % (Table 4). Culture media without PEG_{8000} caused the development of tugescent seedlings. Osmotic water pressures -4 and -8 bars reduced the seedlings turgescences. These results confirmed those of Turner [39].

Rootlets and epicotyls dry matter content were proportional to the OWP and range, respectively, from 7,9 to 13,5 % and from 7,9 to 14,32 %. Under the pressure -0.33 bars, chickpea seedlings had lowest dry matter content. In contrast, under -4 and -8 bars, dry matter content was high (Table 4). Opposite results, found by Turner [39], state that the dry matter content in the aerial and underground parts decreases with increasing the PEG concentration in the culture media and that the aerial part is more affected by the PEG than the root part. Seedlings subjected to water stress undergo a progressive then rapid deterioration. Their fresh and dry weights and their mineral composition were reduced.

The ratio RDMR/EpDMR varied from 0,89 to 0,94 % (Table 4). Results showed that osmotic water pressure levels had similar effects on the two seedlings parts as reported by Daaloul, *et al.,* [10] on durum wheat. However, Jones *et al.,* [19] reported that the dry matter ratio between roots and aerial parts was affected by water deficit.

Rootlets fineness was proportional to the OWP and varied from 77 to 161 mm/g of dry matter. It was low with similar values under low osmotic water pressures -0.33 and -4 bars and significantly higher under the pressure -8 bars (Table 4).

Genotype effect

Very highly significant ($P \leq 1$ ‰) genotypic variability was observed in the rootlets number, the radicle and the epicotyle length, the epicotyle elongation speed and the epicotyle dry matter. It is significant ($P \leq 5$ %) at seedling fresh weight and epicotyle water content and not significant at the ratio RL / EpL, the rootlets water and dry matter content, the report RDMR / EpDMR and the fineness root (Table 3).

Rootlets number per seedling ranged from 0,3 to 4,5. The chickpea genotypes are divided into two interfered homogeneous groups. Genotypes $Beja_1$, $Amdoun_1$, Nayer, Kasseb FLIP96-114C, FLIP88-42C and Chetoui are characterized by a limited rootlets number, whereas Nayer, Bochra FLIP88-42C and have developed a high number (Table 4). These last appear tolerant genotypes osmotic water stress. El Fakhri et al., [14] reported that under water stress conditions, the ability of the plant to maintain a high number of primary roots is considered a good criterion for the water efficiency. Moreover, Sanou and Dabire, [32] indicated that the roots number is a morphological character to consider in the selection programs of water stress resistant genotypes.

Radicle length varies from 4,8 to 54,5 mm. Three interfered homogeneous groups are distinguished. The first one is formed by genotypes $Beja_1$, $Amdoun_1$, Nayer, Kasseb, Bochra and FLIP88-42C which are characterized by long rootlets. The second group is composed of $Beja_1$, $Amdoun_1$, Kasseb, FLIP96-114C and FLIP88-42C whose radicle is moderately long.

Table 3. Mean squares and F test of the vegetative development parameters of the of the chickpea genotypes (*Cicer arietinum L)*.

Variation source	DF	RNb	RL (mm)	EpL (mm)	EpSE (mm/day)	RL/EpL	SFW (g)	RWC (%)	EpWC (%)	RDMR (%)	EpDMR (%)	RDMR / EpDMR	RF ($mm.g^{-1}$)
OWP (bars)	2	30,5***	2550*	1781,3***	6,96***	0,77ns	2,74***	1952220***	1636537***	227,8***	254,2***	0,02ns	50906**
G	7	18,6***	3099***	468,1***	0,87***	1,87ns	0,848*	18369ns	30228*	4,97ns	5,32***	0,06ns	8509ns
Bloc	2	5,1ns	1047ns	30,1ns	0,009ns	2,65ns	0,021ns	12702ns	17532ns	0,46ns	2,92ns	0,006ns	9240ns
OWP*G	14	3,3ns	852ns	209,1*	0,28ns	3,54***	0,973***	18805ns	24939*	3,14ns	4,26*	0,04ns	15489ns
Residue	46	3,2	702	100,3	0,23	1,06	0,321	18502	12606	2,37	1,84	0,04	10278
VC (%)		95	86	59,5	56,3	56,4	58	15,6	12,7	13,5	11,9	23,2	93

DF: Degree of freedom; ns: not significant; *: significant at 5% level; * *: significant at 1 % level; ***: significant at 1 ‰ level; OWP: Osmotic Water Pressure; G: Genotype; VC: Variation coefficient; RNb: Rootlets number; RL: Radicle length; EpL: Epicotyle length; EpSE: Epicotyle speed elongation ; SFW: Seedling fresh weight; RWC: Rootlets water content; EpWC: Epicotyle water content; RDMR: Rootlets dry matter rate; EpDMR: Epicotyle dry matter rate; RF: Rootlets fineness.

Table 4. Means Comparison of the vegetative development parameters of the of the chickpea genotypes (*Cicer arietinum L)* according to the hydrous osmotic pressure and the genotypes;

Parameters		RNb	RL (mm)	EpL (mm)	EpSE (mm/day)	RL/EpL	SFW (g)	RWC (%)	EpWC (%)	RDMR (%)	EpDMR (%)	RDMR / EpDMR	RF ($mm.g^{-1}$)
OWP (bars)	0.33	**3,04a**	**39,9a**	20,3a	**1,39a**	1,97a	**1,28a**	**1195a**	**1172a**	**7,9b**	**7,9b**	0,94a	**77b**
	-4	1,84b	33,4ab	**23,1a**	0,85b	1,62a	1,02a	**661b**	**666b**	**13,5a**	**14,32a**	0,89a	88b
	-8	**0,79c**	**19,7b**	**7,01b**	**0,31c**	1,88a	**0,61b**	755b	808b	12,9a	12,04a	0,89a	**161a**
Genotypes	$Beja_1$	1,7b	29,9abc	10,3b	**0,51c**	**2,34a**	0,80a	901a	**953a**	10,9a	10,52b	0,92a	128a
	$Amdoun_1$	0,7b	19,2abc	9,6b	0,57bc	1,83a	**0,61a**	857a	863ab	11,7a	12,08ab	0,96a	88a
	Nayer	2,6ab	52a	20,2ab	0,92abc	2,03a	1,13a	852a	832ab	12,a	12,03ab	0,98a	106a
	Kasseb	2,1b	44,8ab	22,8ab	1,21ab	2,15a	**1,32a**	**793a**	**783b**	**12,6a**	**12,54a**	**1,00a**	150a
	Bochra	**4,5a**	**54,5a**	**27,8a**	**1,28a**	2,23a	1,29a	877a	867ab	11,1a	11,43ab	**0,80a**	**152a**
	FLIP96-114C	0,4b	11,1bc	13b	0,65abc	**1,10a**	0,63a	840a	900ab	11,7a	11,43ab	0,80a	87a
	FLIP88-42C	2,8ab	31,4abc	21,9ab	1,07abc	1,67a	1,26a	**940a**	915ab	**10,3a**	10,85ab	0,81a	81a
	Chetoui	**0,3b**	**4,8c**	**9,1b**	0,56bc	1,25a	0,74a	903a	945ab	10,8a	**10,45b**	0,95a	**77a**

Numbers of the same column accompanied by the same letter are not significantly different (Student-Newman and Keuls test , $P \leq 5$ %); OWP: Osmotic Water Pressure; RNb: Rootlets number; RL: Radicle length; EpL: Epicotyle length; EpSE: Epicotyle speed elongation ; SFW: Seedling fresh weight; RWC: Rootlets water content; EpWC: Epicotyle water content; RDMR: Rootlets dry matter rate; EpDMR: Epicotyle dry matter rate; RF: Rootlets fineness.

The last group is composed of $Beja_1$, Amdoun1, FLIP96-114C, FLIP88-42C and Chetoui who presented short radicles (Table 4). Several authors give a particular attention to a deep rooting permitting look for water in the deeper soil layers even if this depth is reached by only one principal root [14]. Subbarao *et al.,* [30] reported that root system capable of extracting soil water is an essential characteristic for drought resistance. Turner [38] confirmed this genotypic variability for the length of the radicle. He underlined that the increase in the roots weight indicates a greater density or more significant roots length. These two parameters are good indicators of morphological adaptation to water deficit. Each variety has adapted to water shortage, according to the stress severity, by varying morphology, branching and extending its root system [34]. In fact, the water amount absorbed by the plant depends on root system characteristics such as the new produced roots number, the roots size and length, the vascular tissues differentiation model and the root age [24]. In addition, Fischer *et al.,* [15] noted that, under water stress conditions, selection for root mass leads to a significant increase in grain yield, whereas selection for increased root length is more interesting in the case of a severe stress. Opposite results were found by Boubaker and Yamada [6] which report that, under water stress conditions the genotypic difference in wheat root length is not significant. Dib *et al.,* [11] showed that the rooting characteristics are genetically controlled.

Epicotyle length varies from 9,1 to 27,8 mm. Mean comparisons showed two overlap homogeneous groups. The first includes genotypes Bochra, Nayer, Kasseb and FLIP88-42C that have developed long epicotyles. The second group is composed of Chetoui, FLIP96-114C, $Beja_1$, $Amdoun_1$, Nayer, Kasseb and FLIP88-42C which are characterized by short epicotyles (Table 4). Sané *et al.,* [31] showed that water stress induced by PEG on *in vitro* culture of two genotypes of palm date trees, reduced the length of the epicotyle and that the most tolerant genotype has developed the longest epicotyle.

Epicotyle speed elongation varies from 0,51 to 1,28 mm/day. Genotypes can be divided into three interfere homogeneous groups. Genotypes: $Amdoun_1$, Nayer, Kasseb Bochra, FLIP96-114C and FLIP88-42C are vigorous and accelerated growth of their epicotyles. The second group includes genotypes $Amdoun_1$, Nayer, Kasseb, FLIP96-114C, FLIP88-42C and Chetoui which showed less accelerated epicotyles growth. Genotypes $Beja_1$, $Amdoun_1$, Nayer, FLIP96-114C, FLIP88-42C and Chetoui are stunted and showed a slower growth of their epicotyles (Table 4). Boubaker and Yamada [6] reported that under water stress conditions, the seeds ability to produce vigorous plants indicates that they have a genetic potential for tolerance to water stress.

Chickpea genotype seedlings fresh weight varied from 0,61 to 1,32 g. Mean comparisons (SNK test, $P \leq 0.5$ %) showed a single homogeneous group. However, genotypes Nayer, Kasseb, Bochra and FLIP88-42C appear more vigorous and fresh weight were relatively higher than those of $Beja_1$, $Amdoun_1$, Chetoui and FLIP96-114C (Table 4). Wery *et al.,* [40] reported that good plant vigor was significantly associated with tolerance to drought. Toker [37] suggested that the early plant vigor and strength could be adopted as a criterion for drought tolerance in chickpea. According Pacucci, *et al.,* [27], under drought conditions, plant vigor and high dry matter production could be considered among the main selection criteria for improving the chickpea yield performance. Muhammad and Iram [25] found that water deficit has a significant inhibitory effect on roots fresh and dry weights, above bean ground biomass and nodules (*Phasiolus vulgaris* L.). Under severe water stress conditions, differences existing between genotypes were highly significant, whereas under low stress conditions are not significant [6].

Epicotyle water content and dry matter content are inversely proportional. They ranged, respectively, between 783-953 % and 10,45 to 12,54 % (Table 4). Mean comparisons permit to classify chickpea genotypes into two overlap homogeneous groups. The epicotyles of the genotypes $Beja_1$, $Amdoun_1$, Nayer, Bochra, FLIP96-114C, FLIP88-42C and Chetoui are turgid water but contain small amounts of dry matter. Conversely, genotypes Kasseb, Amdoun1 and Nayer have epicotyles rich in dry matter and less turgid water (Table 4).

Interactions between factors

The interaction (G x OWP) is highly significant ($P \leq 1$ ‰) for the ratio RL/EpL and the seedlings fresh weight, significantly ($P \leq 5$ %) for the length, the water content and the dry matter content of the epicotyle and not significant for the remaining variables (Table 3). These results indicate that chickpea cultivars differ in their sensitivity to osmotic water stress and specified parameters can be chosen as

selection criteria for tolerance to osmotic water stress [6].

The report RL/EpL varies according to chickpea genotypes and OWP from 0 to 3,79. Under different OWP, genotypes Nayer and FLIP96-14C presented similar reports. Under the OWP -8 bars, genotypes FLIP88-42C and Chetoui developed epicotyles longer than the radicle; in reverse, Nayer, Kasseb and Bochra produced longer radicle than epicotyles. This characteristic conferred on these genotypes tolerance to osmotic water stress. In fact, under water deficit conditions, the development of a deep root system is very required.

Seedlings fresh weight varies from 0,33 to 2,31 g/seedling. Under OWP -8 bars, genotypes Nayer, Kasseb, Bochra, FLIP96-114C and FLIP88-42C have recorded the lowest seedlings fresh weight, while germination of $Beja_1$ and $Amdoun_1$ is completely inhibited. Under the OWP -0.33 and -4 bars, genotypes $Amdoun_1$, Bochra, FLIP96-114C and FLIP88-42C developed seedlings with similar fresh weight. Turner [39] reported that water stress reduced the seedlings fresh weight and their mineral compositions.

Epicotyle length varied simultaneously depending chickpea genotypes and OWP from 0 to 47,37 mm. Genotypes Bochra, FLIP96-114C and FLIP88-42C showed epicotyles longer under -4 bars that under -0,33 bars. Osmotic water pressure -8 bars seems very high. It inhibited the growth of the chickpea genotypes epicotyles, in particular, those of $Beja_1$, $Amdoun_1$ and FLIP88-42C which are reduced to zero. Under this same OWP, genotypes Nayer, Kasseb, Bochra and FLIP88-42C developed similar epicotyles lengths.

Epicotyle water content varies from 479 to 1226 %. The highest contents are recorded under the OWP -0,33 bars. Under -8 bars the tissue turgor of Bochra, FLIP88-42C and Chetoui is higher than under -4 bars. Nayer and Kasseb showed similar water turgescences under -4 and -8 bars. Turner [39] indicated that turgor in water plant organs is negatively affected by high PEG concentrations. Blum [5] noted that water deficit often leads to a decrease in cell turgor, which limits the growth of tissues. Grieu [17] noted that the performance of plants subjected to water stress depend on their ability to accumulate water. Under water stress the maintenance of the tissue turgor contributes to limiting the negative effects of water stress on stomata conductance and photosynthesis [23], cell expansion [9] and plant growth [2]. This ability gives the plant a better tolerance to water internal deficit [21].

Epicotyle dry matter content varies from 7,53 to 17,3 %. It should be noted that under the OW P -0.33 bars, eight chickpea genotypes accumulated similar and very low dry matter contents. While, the highest contents are accumulated by $Amdoun_1$ and FLIP96-114C under the OWP -4 bars. Under this OWP, Nayer, Kasseb, Bochra and FLIP88-42C accumulated similar and high epicotyle dry matter content. These results are in contradiction with those of Turner [39] and Zhang *et al.,* [42] who reported that water stress reduced seedlings dry weight.

Tolerance index to osmotic water stress

Chickpea genotypes showed variable responses to osmotic water stress induced by PEG_{8000}. The identification of tolerant genotypes to such abiotic stress, using germination and vegetative development parameters, seems confused and unsatisfactory. It would be useful to use the tolerance index to osmotic water stress. Parameters that presented genotypic variability are: germination rate, time and energy, rootlets number per seedling, radicle and epicotyle length, epicotyle speed elongation, seedling fresh weight, epicotyle water and dry matter contents. Three groups of genotypes were distinguished. Genotypes Nayer and Kasseb appeared highly tolerant to osmotic water stress; while, $Amdoun_1$, $Beja_1$ and FLIP96-114C were the most sensitive. Cultivars Bochra, FLIP88-42C and Chetoui were moderately sensitive to osmotic water stress (Table 5). These results reflect a wide genotypic variability of these chickpea cultivars toward the osmotic water stress. In fact, Toker [37] noted that the genotype Chetoui is sensitive to water stress. On the contrary, Slim *et al.,* [34] indicated that varieties Bochra and Chetoui proved to be the most tolerant to water stress, while Kasseb and Nayer were the most sensitive to water deficit.

4. Conclusion

Osmotic water stress, induced by PEG_{8000}, has negatively affected germination and vegetative development parameters of chickpea seedlings. Germination was more accelerated, on filter paper than on agar, with higher germination rate, germination energy and seedlings vigorous development. Nevertheless on filter paper, the OWP -8 bars appeared as very high. It completely inhibited seed germination. On agar, it resulted in very low germination rate and stunted and malformed seedlings. A wide genotypic variability of the tested chickpea cultivars to osmotic water stress was revealed.

Genotypes Nayer and Kasseb were highly tolerant to osmotic water stress; while $Amdoun_1$, $Beja_1$ and FLIP96-114C were the most sensitive. Cultivars Bochra, FLIP88-42C and Chetoui were moderately sensitive.

In vitro screening of chickpea genotypes for water stress tolerance, based on germination and seedling vegetative development is very informative. It could be a preliminary step in a rational screening consolidated research *in situ*.

Table 5. Tolerance index to osmotic water stress of the chickpea genotypes (*Cicer arietinum* L).

Genotypes	GR (%)	GT (days)	GE (seeds/day)	NbR	RL (mm)	EpL (mm)	EpSE (mm/day)	SFW (g)	EpWC (%)	EpDMR (%)	TIOWS
$Amdoun_1$	0,036	1,054	0,022	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,111
$Beja_1$	0,324	3,106	0,103	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,353
FLIP96-114C	0,045	1,919	0,014	1,000	0,206	0,000	0,000	0,548	0,000	0,000	0,373
Bochra	0,203	1,131	0,113	0,309	0,547	0,473	0,284	0,019	0,663	1,442	0,518
FLIP88-42C	0,338	1,194	0,183	0,071	0,085	0,851	0,550	0,129	0,596	1,597	0,559
Chetoui	0,160	2,757	0,035	0,000	0,000	0,323	0,052	0,214	0,621	1,522	0,568
Nayer	0,367	1,688	0,178	0,870	1,293	0,497	0,360	0,141	0,510	1,785	0,769
Kasseb	0,324	2,678	0,129	0,345	1,088	0,471	0,428	0,052	0,516	1,786	0,782

GR: Germination rate; GT: Germination time; GE: Germination energy; RNb: Rootlets number; RL: Radicle length; EpL: Epicotyle length; EpSE: Epicotyle speed elongation ; SFW: Seedling fresh weight; EpWC: Epicotyle water content; EpDMR: Epicotyle dry matter rate; TIOWS: Tolerance index to osmotic water stress.

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