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SCREENING OF OSMOTIC WATER STRESS TOLERANT CHICKPEA GENOTYPES (*CICER ARIETINUM* L.) ON THE BASIS OF GERMINATION PARAMETERS AND ACCUMULATED SOLUBLE SUGARS AND PROLINE CONTENT

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Abstract: Eight kabuli chickpea genotypes (*Cicer arietinum* L.) Beja$_1$, Amdoun$_1$, Nayer, Kasseb, Bochra, FLP96-114C, FLP88-42C and Chetoui were germinated, *in vitro culture*, on Whatman n° 2 filter paper media at four osmotic water pressures: -0,33; -4, -6 and -8 bars as induced by polyethylene glycol (PEG $_{8000}$). Osmotic water stress negatively affected germination parameters and enhanced soluble sugars and proline accumulation. Broad genotypic variability of the chickpea cultivars was revealed with respect to osmotic water stress. At -0,33 bars, germination occurred at a high rate and exhibited elevated germinative energy. On the other hand, OWP -8 bars proved too high, as it completely inhibited chickpea germination. Soluble sugars and proline accumulation were proportional to osmotic water pressures. Hierarchical cluster analysis revealed that under high OWP (-6 bars), genotypes Beja$_1$, Amdoun$_1$, Nayer, FLIP96-114C and Chetoui were sensitive to the osmotic water stress; whereas, Kasseb, Bochra and FLIP88-42C were tolerant.
Keywords: Chickpea; Polyethylene glycol$_{8\,000}$; Germination; Soluble sugars; Proline.

INTRODUCTION

Seed germination, resulting in radical emergence and development (Dirik 2000) is an important physiological stage of the plant vegetative cycle (Khouja et al., 2002). It determines the future establishment of plants and is a useful predictor for successful culture. Boubaker and Yamada (1995) found that germination in dry soil produces plants with low vigor. The ability of seeds to produce vigorous plants under water deficit conditions indicates a genetic potential for tolerance to water stress. Blum and Ebercon (1981) indicated that under water stress conditions, seed germination and plant growth are a quick and reliable way to identify drought tolerant genotypes. The physiological effects of drought lead to osmotic water stress in the plant (Sané *et al.,* 2005). Polyethylene glycol (PEG), a non-ionic hydropolymer that does not rapidly enter in plant tissues, is widely used to induce osmotic water stress (Sané *et al.,* 2005). Erskine *et al.,* (1994) evaluated, in situ, wheat genotypes for drought tolerance by examining differential growth under osmotic water stress induced by PEG. Sané *et al.,* (2005) found that the use of PEG in, in vitro culture, allows quick and easy identification of genotypes tolerant to water stress. This technique has been commonly used to assess the level of tolerance of wheat cultivars to drought (Dirik 2000).

Osmotic adjustment essential to maintain tissue turgor is critical for various vital functions of the plant (Slim et al., 2006). It appears as a major mechanism of plants ability to adapt to water stress (Zhang et al., 1999). Morgan (1984) stated that osmotic adjustment is an important physiological adaptation to reduce, to a

minimum, the harmful effects of water deficit and protect membranes and enzyme systems (Santarius, 1973), especially of the juvenile organs. A Plants ability to make osmotic adjustment is due to the active accumulation of solutes in the symplast. In the event of water stress, solute content increases significantly, decreasing the osmotic potential and contributing to osmotic adjustment (Belhassen et al., 1995). Generally, these solutes are inorganic ions such as potassium (Gaudillière et Barcelo, 1990) and nitrate (Pedersen et al., 1996), soluble sugars (Munoz *et al.,* 1998), amino acids such as proline (Newton et al., 1986), abscisic acid and organic acids such as malic acid (Curtis, 2004). Production of different types of organic and inorganic solutes is one of the largest plant responses toward drought and other abiotic stresses (Ashraf and Harris 2004). Soluble sugars act as agents which maintain the osmotic cell turgor and as osmoprotectres that stabilize proteins and cell membranes (Ingram and Bartels, 1996). Following abiotic stress such as drought, proline accumulation in the plant has variables effects. Its role as an osmoticum has been reported by many authors (Kauss, 1977). Kouakou *et al.,* (2008) noted that it represents one of the most notable expressions of water and osmotic stresses.

Aspinall and Paleg (Aspinall and Paleg 1981) announced that, in plants subjected to drought; this amino acid plays multiple physiological roles, in particular, osmoregulation which limits energy and nitrogen losses and as senescence signal. Venekamp *et al*., (1989) indicated that accumulation acts as an osmoticum in the cytosol and the vacuole, protects membranes and enzyme systems and regulates pH. Karamanos *et al*., (1983) found that increasing amounts of proline in the tissues may be associated with significant tissue dehydration and more effective mechanisms to prevent drought. Savitskaya, (1976) noted that proline synthesis was associated with hydrolysis of proteins induced by water deficit. According to Mefti *et al*., (2001), accumulation of organic solutes such as proline, is simply a phenomenon of adaptation to drought, allowing the plant to maintain turgor by reducing its water potential, which is a form of osmotic adjustment potential. This type of tolerance allows the plant to ensure its normal physiological functions despite his deteriorating internal hydrous state due to drought (De Raissac, 1992). On the other hand, Hanson *et al*., (1977) reported by Zid and Grignon (1991), indicated that the accumulation of proline is not an adaptive response to stress, but rather the sign of a metabolic disturbance. Bellinger *et al*., (1991) announced that proline physiological role is poorly understood and that this accumulation does not indicate sensitivity or resistance. Many traits are involved in tolerance to water stress and the use of physiological parameters other than proline accumulation seems recommended (Bellinger *et al*., 1991). However, selection for tolerance to water stress based on the accumulation of proline must necessarily be empirical.

Vachon *et al.,* (2005) reported that classification analysis objectives are to identify classes within mixed entities that are believed to belong to different populations. Blashfield and Aldenderfer (1988) suggested that about two-thirds of all uses applied classification analyzes involved the agglomeration method, or "hierarchical cluster analysis". This analysis can produce as many classes as there are entities in the database (Milligan, 1981). Initially, each entity is a subclass. These subclasses are then grouped based on their similarity to each entity that is part of group (Blashfield RK and Aldenderfer, 1988).

MATERIALS AND METHODS

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, (Table 1) were grown *in vitro* culture with the aim to evaluate their tolerance to osmotic water stress induced by PEG_{8000}.

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 performed on two layers of filter paper Whatman n° 2 in Petri dishes (Dirik 2000). Four osmotic water pressures (OWP) -0,33; -4; -6 and -8 bars

were induced by the addition of the respective doses of PEG_{8000}: 0; 16,75; 19,5 and 23 g/100 ml of distilled water. Ten seeds per genotype were sown in each Petri dish following a split block experimental design, with three replications. Germination conditions were 22 °C temperature, 70 to 80% relative humidity, 2500 lux luminous intensity and 10/14h daily photoperiod (Tahri, *et al.,* 1998). Seeds were considered germinated when the radicles pierced the seed coat or were clearly elongated (Come, 1970).

Table 1. Pedigree and origin of the studied kabuli chickpea genotypes (*Cicer arietinum* L.)

S.No.	Name	Pedigree	Origin
1	Beja $_1$	INRAT 93-1	Tunisia
2	Amdoun$_1$	Be-sel-81-48	Tunisia
3	Nayer	FLIP 84 - 92 C	Tunisia
4	Kasseb	FLIP 84 - 460 C	Tunisia
5	Bochra	FLIP 84 - 79 C	Tunisia
6	FLP96-114C	X93 TH 74/FLIP87-51CXFLIP91-125C	ICARDA/ICRISAT
7	FLP88-42C	X85 TH 230/ILC 3395 x FLP 83-13C	ICARDA/ICRISAT
8	Chetoui	ILC3279	Tunisia

Soluble sugars content

Four samples of 20 to 30 mg of fresh matter (FM) from epicotyles and radicals were placed in 10 ml of boiling water for 15 min. After centrifugation at 10 000 rpm at 4 °C for 10 min, the supernatant was collected and the volume was adjusted to 50 ml. Content of soluble sugar water was determined using the Dubois *et al*., (1956) method as modified by Buysse and Merckx (1993). One ml of the supernatant was placed in a test tube to which was added 1 ml of 18 % phenol and 5 ml of concentrated sulfuric acid. The mixture was shaken and the absorbance was determined by spectrophotometer at 490 nm optical density.

Proline dosage

Proline was assayed according to the Chinard (1952) method. One hundred mg of fresh matter from epicotyls and radicals of each sample were placed in a test tube and cut into pieces. They were homogenized in 10 ml of Sulfosalicylic acid aqueous solution at 3 % concentration and filtered through a filter paper Whatman n° 2. In a test tube of 20 ml capacity, 2 ml of the filtrate was reacted with 2 ml of ninhydrin and 2 ml of glacial acetic acid. Samples were heated for 1 hour in a water bath at 100 °C. To stop the reaction, samples were placed on ice. Four ml of toluene were added to the samples. The whole mixture was vigorously stirred for 10 to 15 seconds. After standing for 20 min, the optical density of the toluene portion was determined using a spectrophotometer at 520 nm optical density.

Counts and measurements

- Germination Rate (GR, %): The percentage of the germinated seeds per Petri dish;
- Germination time or germination speed (GT, days): The number of days to seed germination. It has been defined by Dirik, (2000) according to the formula:

$$GT = \sum_j^i (n_i \times t_i) / N$$

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

- Germinative energy or germinative value (GE, Seeds/day) as defined by Djavanshir and Pourbeik, (1976). It is the inverse of the germination time and indicates the number of germinated seeds per day.
- Soluble sugars content (SS, $mg.g^{-1}$ FM) accumulated in seedlings grown on filter paper;
- Proline content (Pr, $\mu mol/g^{-1}$ FM) accumulated in seedlings grown on filter paper;

Results were processed by statistical analyzes, including ANOVA, means comparison (Student-Newman-Keuls (SNK) test $P \leq 0.5$ %) and hierarchical cluster analysis based on Euclidean distance and Ward's method (Sokal and Sneath, 1963).

RESULTS AND DISCUSSION

Germination parameters

Variance analysis showed very highly significant differences (P≤1 ‰) among OWP, genotype and the interactions (OWP x Genotype) for the germinations rate and energy and time. Variation coefficients were 10,27; 12,27 and 11,2 % , respectively for germination rate, germination energy and germination time (Table 2). These results indicate that chickpea cultivars differ in their sensitivity to osmotic water stress. Boubaker and Yamada (1995) suggested that differences in the response of wheat cultivars to osmotic water stress can be attributed to differences in structural or physiological traits such as osmoregulation capacity and integrity of cell membranes that allow maintain a relatively high water potential.

Table 2 Mean squares and F test of the germinations rate, energy and time and soluble sugar and proline contents of the chickpea genotypes (*Cicer arietinum* L).

Variation Source	GR (%)	GE (seeds/day)	GT (days)	SS (mg.g-1 of the FM)	Pr (µmol.g-1 of the FM)
OWP	53492,71***	29,63***	11,543***	134,75***	131951***
Genotype	378,42***	0,373***	0,978***	2,13**	4558*
Bloc	7,29ns	0,003ns	0,027ns	2,04*	6841*
OWP*Genotype	184,77***	0,195***	0,682***	2,09***	1723ns
Error	52,453	0,039	0,269	0,47	1998
VC (%)	10,27	12,27	11,2	23,5	41

Abb.: ns: non significant; *: significant at 5 % level; * *: significant at 1 % level; ***: significant at 1‰ level;; GR: Germination rate; GE: Germination energy; GT: Germination Time; SS: Soluble sugar content; Pr: Proline content; OWP: Osmotic water pressure; CV: Variation Coefficient.

Feutry and Bertrand (2003) reported that germination rate and time were important criteria that characterize the speed and uniformity of seed germination. Boubaker and Yamada (1995) have proposed that the germination parameters can be used as selection criteria for tolerance to osmotic water stress. Germination rates varied from 0 to 100 %, inversely proportional to the OWP. Means comparison (SNK test, P ≤ 0,5 %) showed that there are three homogeneous groups (Table 3). The first one is composed of the OWP -0.33 bars which were associated with higher germination rates. The second group includes OWP -8 bars that inhibited germination of all seeds and zero germination. The third group, associated with OWPs of -4 and -6 bars, presented with similar intermediate germination rates (Table 4). Dirik (2000) and Jaouadi *et al*., (2010) noted that the increase in osmotic water stress of the culture substrate greatly decreased the germination rate and negatively affected germination capacity, of Lebanon Cedar and *Acacia tortilis* seeds respectively.

Germination energy varied from 0 to 2, 5 seeds/day. It is inversely proportional to OWP. Means comparisons (SNK test, P≤0,5 %) revealed four distinct homogeneous groups (Table 3). Under OWP -8 bars germination energy is zero. On the other hand, it reaches a maximum value in the absence of PEG_{8000}. Under OWP -4 and -6 bars, germination energy reached intermediate and distinct values. Turner (1986) indicated that the germinative capacity or energy is inversely proportional to the PEG_{8000} concentration in the media culture. Evolution of this parameter according to time shows the negative action of the PEG_{8000} on the germination latency.

Table 3. Mean comparisons of germinations rate, energy and time and soluble sugars and proline contents of the chickpea genotypes (*Cicer arietinum* L.) according to osmotic water pressures (OWP)

OWP	GR (%)	GE (seeds/day)	GT (days)	SS (mg.g-1 of FM)	Pr (µmol.g-1 of FM)
-0,33bars	100c	2,5d	4a	0,341a	58,9a
-4bars	92,1b	2,13c	4,6b	5,005c	193,7b
-6bars	90b	1,83b	5,34c	3,399b	72,8a
-8bars	0a	0a	-	-	-

Abbs.: GR: Germination rate; GE: Germination energy; GT: Germination Time; SS: Soluble sugar content; Pr: Proline content; OWP: Osmotic water pressure; Numbers of the same column accompanied by the same letter are not significantly different (SNK test, P=0,5 %); Numbers bold are the extreme values.

Germination time varies from 4 to 5, 34 days, increasing in proportion to OWP. Means comparisons showed three distinct homogeneous groups (Table 3). The first one is composed by the OWP -0.33 bars which allowed rapid germination. The second group is comprised of the OWP -6 bars with slower germination than OWP -4 bars (Table 3). Dirik (2000) found that the germination time is slightly higher in stressed treatments compared to unstressed treatments. Jaouadi *et al*., (2010) noted that the germination time is proportional to the osmotic water stress intensity. Germination rate varies according to chickpea genotypes from 60.8 to 75 %. Means comparison revealed considerable overlapping (Table 4). Genotypes; Nayer, Kasseb, Bochra, FLIP96-114C, FLIP88-42C and Chetoui characterized by high and similar germination rates some of these could not be separated from those classified with medium germination rates and some of those overlapped all but the slowest germinating genotype. $Beja_1$, Kasseb, Bochra, FLIP96-114C, FLIP88 - 42C and Chetoui showed similar and medium germination rates. The last group is formed by $Beja_1$, $Amdoun_1$, Kasseb, FLIP96-114C, FLIP 88 - 42C and Chetoui which were distinguished by the lowest germination rates (Table 4).

Table 4. Mean comparisons of the germination rate, germinative energy and germination time of the chickpea genotypes (*Cicer arietinum* L.) according to the genotypes

Genotype	GR (%)	GE (seeds/day)	GT (days)	SS (mg.g-1 of FM)	Pr (µmol.g-1 of FM)
$Beja_1$	62,5ab	1,42ab	4,72ab	3,308b	117,63ab
$Amdoun_1$	60,8a	1,33a	5,01b	2,921b	94,66ab
Nayer	75c	1,71cde	4,6ab	2,900b	119,83ab
Kasseb	74,2abc	1,81de	4,17a	2,601b	109,13ab
Bochra	75bc	1,84e	4,13a	2,902b	66,02a
FLIP96-114C	72,5abc	1,58bc	4,94b	3,3467b	145,04b
FLIP 88 - 42C	72,5abc	1,63bcd	4,79ab	3,410b	108,55ab
Chetoui	71,7abc	1,6bcd	4,81ab	1,928a	107,16ab

Abbs.: GR: Germination rate; GE: Germination energy; GT: Germination Time; SS: Soluble sugar content; Pr: Proline content; FM: fresh matter; Numbers of the same column accompanied by the same letter are not significantly different (SNK test, P=0,5 %); Numbers bold are the extreme values.

Germinative energy varies, according to chickpea genotypes, from 1,33 to 1,84 seeds/day. Means comparison ranked the genotypes into a series of high to low GE values (Table 4). The first group is composed of genotypes $Beja_1$ and $Amdoun_1$ which showed the lowest germination energy. In contrast, genotypes Nayer, Kasseb and Bochra presented the highest germination energy. Germination time shown by chickpea genotypes ranged from 4,13 to 5,01 days. Means comparison showed two overlapped high and two low genotypes, with the others all intermediate (Table 4). Germination rate varies simultaneously according to OWP and chickpea genotypes from 0 to 100 %. It is inversely proportional to the OWP. Means comparisons showed three overlapping groups. Under -8 bars OWP no chickpea genotypes germinated. In contrast, under the OWP -0.33 bars the germination rate is maximum for all genotypes. Under OWP -4 and -6 bars genotypes Nayer, Kasseb Bochra, FLIP96-114C, FLIP 88 - 42C and Chetoui attained similar intermediate values of

germination rates (Figure 1a). Germination energy varied from 0 to 2,5 seeds/day, inversely proportional to the OWP. Means comparison showed three overlapping ranges. The highest germinative energy is recorded by all genotypes in the OWP -0.33 bar treatment Under the OWP -4 and -6 bars chickpea genotypes showed intermediate and similar values of germination energy (Figure 1b). Germination time varies from 4 to 6,19 days. It is proportional to osmotic water pressure. Means comparison showed three interfered homogeneous groups. Under the OWP -0.33, bars, all chickpea genotypes have put very short and similar values of germination time. Under the OWP -4 bars, genotypes Kasseb and Bochra have germination time equivalent to those under the OWP -0.33 bars. Under the OWP -4 and -6 bars, genotypes have much more time to germinate (Figure 1c).

Soluble sugars accumulation

Variance analysis showed very highly significant differences (P ≤ 1 ‰) among OWP and the interactions (OWP x Genotype) and highly significant (P≤1%) genotypic variability on the soluble sugars accumulation (Table 2). These results indicate that the ability of chickpea genotypes to accumulate soluble sugars is affected by the osmotic water pressure. Mefti *et al*., (2001) detected a significant (P ≤ 5 %) genotypic variability of *Medicago truncatula* (L.) Gaertn. The interaction (Populations x Water treatments) has very highly significant effects on the soluble sugars accumulation. It appears that stressed plants have responded by increasing the soluble sugars content, which is actually an adjustment factor to water stress conditions for the maintenance of highly cellular integrity. Soluble sugars contents vary across OWP values ranging from 0,341 to 5,005 mg g^{-1} FM. Mean comparison showed three different groups. Higher soluble sugar content accumulated under the OWP -4 bars, whereas the lowest was recorded in the absence of PEG_{8000}. In the OWP -6 bars the soluble sugars accumulation took an intermediate value (Table 3). Hooda *et al*., (1999) found that in drought conditions, soluble sugars cellular concentration increased in the nodules of *Vigna radiata*. The application of water stress on millet ecotypes caused an increase in the soluble sugars content proportional to the intensity of water stress (Radhouane, 2011) and osmotic adjustment (Crowe *et al.,* 1992). Zerrad *et al*., (2006) noted that increase in soluble sugars content in roots and coleoptiles of durum wheat seedlings is increasingly distinct that the water stress intensity increases. They reported that soluble sugars contribute in the maintenance of the osmotic balance force to keep the cytosol turgor and volume as high as possible and permit also to preserve membrane integrity of dried organs and protect proteins.

Soluble sugars contents in chickpea genotypes ranged from 1,928 to 3,41 mg.g^{-1} FM (Table 4). Two groups are highlighted. The first group is composed of genotypes $Beja_1$, $Amdoun_1$, Nayer, Kasseb, Bochra, FLIP88-114C and FLIP96-42C that have accumulated high levels of soluble sugars. Chetoui accumulated significantly lower levels of SS (Table 4). Soluble sugars contents accumulated in the tissues concordantly with OWP and chickpea genotypes from 0,2 to 6,41 mg.g^{-1} FM. Means comparison showed five interfered homogeneous groups. The highest levels are synthesized in the OWP -4 bars, particularly, by genotypes $Beja_1$, FLIP88-114C and FLIP96-42C, whereas the lowest are synthesized in the germs developed in the absence of PEG_{8000}. It should be noted that under the OWP -6 bars soluble sugars accumulation is lower than under the OWP -4 bars (Figure 2a). Laouar *et al*., (2001) found that under intense water stress Medicago populations have accumulated significant soluble sugars contents. They reported that increasing of the concentration of this osmoticum is actually an adaptation parameter to water stress (Kameli and Losel, 1995), which can provide a guarantee for maintaining a high cellular integrity (Ben Salem 1993).

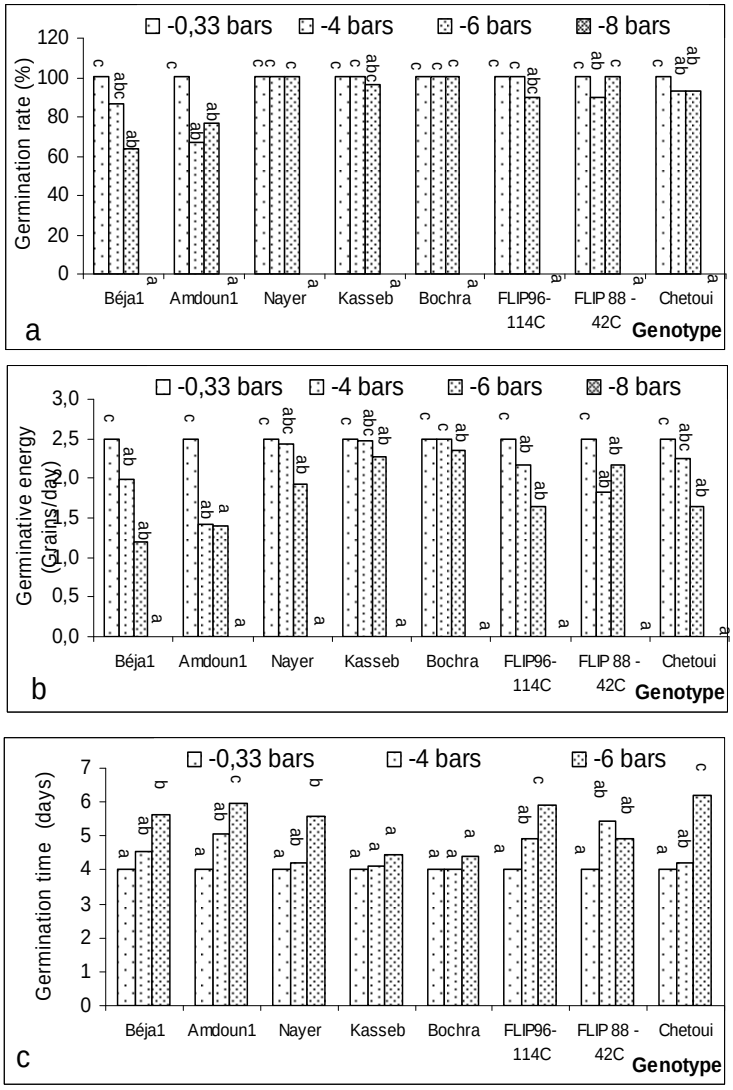

Figure 1. Variations of the germination rate (a), germination energy (b) and germination time of the chickpea genotypes (*Cicer arietinum* L.) according to the interaction (Genotype x Water pressure) (bars of the same histogram accompanied by the same letters are not significantly different; SNK test; P = 5 %).

Proline accumulation

Variance analysis showed that OWP effects were very highly significant (P<1 ‰), genotypic variability is significant (P<5 %) and interaction (OWP x Genotype) is not significant on the proline accumulation (Table 2). Facing to osmotic water stress caused by the PEG_{8000}, chickpea genotypes showed similar behavior at the proline accumulation. These results are consistent with those of Zhang and Archbold (1993), who observed that proline, was not detected in certain vegetable species water stressed. However, Radhouane, (2011) having found an interaction (millet ecotype x water treatment) for significant proline accumulation, stipulates that each population of millet behaved differently to water stress. Proline contents vary, according to OWP from 58,9 to 193,7 µmol.g^{-1} of FM. Means comparison showed two different homogeneous groups (Table 3). The first group represents the OWP -0.33 and -6 bars, in which proline accumulation is low with similar values. The second group is composed of the OWP -4 bars resulted in a higher proline accumulation. These results are consistent with those of Mefti *et al*., (2001) who noticed that water stress caused a significant increase in the accumulation of proline in the leaves of *Medicago truncatula* (L.) Gaertn. Zerrad *et al*., (2006) found that the increase in the quantity of proline in durum wheat seedlings leaves is positively correlated with the duration

and degree of water stress. For its part, Radhouane (2011) consigned a severe water stress resulted in large accumulations of proline, while a moderate water stress did not result in significant changes of this substance. Proline concentration in chickpea genotypes varies from 66,02 to 145,04 µmol.g^{-1} of FM (Table 4). Two strongly overlapped homogeneous groups were highlighted. The first includes genotypes Beja$_1$, Amdoun$_1$, Nayer, Kasseb, FLIP96-114C, FLIP88-42C and Chetoui who have accumulated high levels of proline. Genotypes Beja$_1$, Amdoun$_1$, Nayer, Kasseb Bochra, FLIP88-42C and Chetoui form the second group and have accumulated lower levels of proline (Table 4). Radhouane (2011) found a genotypic variability in millet for the proline accumulation. *In vitro* culture, PEG resulted in an increase in the proline concentration in the callus of resistant durum wheat cultivars (Bajji *et al*., 2000). Zid and Grignon (1991) have suggested that genotypic variability in the potential for the proline accumulation induces the possibility of selection for this trait. Muhammad and Iram (2005) reported that proline accumulation induced by abiotic stress is a better indicator of drought tolerance. Singh *et al*., (1973) proposed to use this parameter as a criterion for drought tolerance. For screening for resistant genotypes to water deficit, Benlarabi Monneveux (1988) and Bellinger *et al*., (1989) used the capacity of the proline accumulation, respectively, in durum wheat and in maize. Proline contents vary simultaneously according to OWP and the chickpea genotypes from 34,5 to 242,3 µmol.g^{-1} FM. Means comparison revealed four interfered homogeneous groups. The highest contents of proline are synthesized in the OWP -4 bars. Whereas under the OWP -0,33 and -6 bars the proline contents are low and similar (Figure 2b). Genotypes Beja$_1$, Nayer, FLIP96-14C and FLIP88-42C have accumulated the maximum proline at the OWP -4 bars. They seem more tolerant to osmotic water stress.

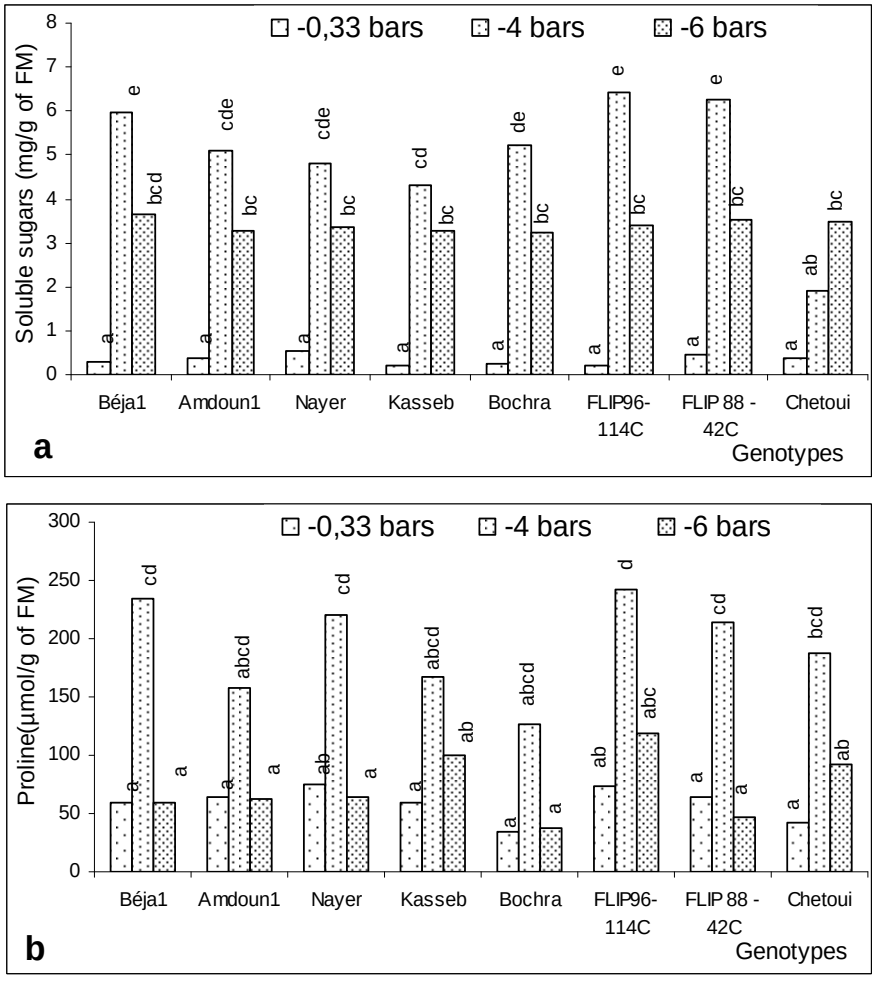

Figure 2. Variations of the soluble sugars (a) and proline (b) contents accumulated by the chickpea genotypes (*Cicer arietinum* L.) according to the interaction (Genotype x Water pressure) (bars of the same histogram accompanied by the same letters are not significantly different; SNK test; P = 5 %).

Hierarchical cluster analysis

Hierarchical cluster analysis based on quantitative parameters revealed heterogeneity in the behavior of chickpea genotypes toward the osmotic water pressures. Three groups of genotypes were identified based on the Euclidean distance and Ward's method. Euclidean distance or dissimilarity level (d) ranged from 0,01 to 47,85 (Figure 3). The first group is at 0,26 level of the dissimilarity. It is composed of eight genotypes: $Beja_1$, $Amdoun_1$, Nayer, Kasseb Bochra, FLIP96-114C FLIP88-42C and Chetoui under the OWP -0.33 bars (Figure 3). In the culture media saturated state, all chickpea genotypes exhibited rapid germination with maximum rates and energy and low accumulation of soluble sugars and proline (Table 5). Under the OWP -0.33 bars, discrimination between chickpea genotypes appears too difficult.

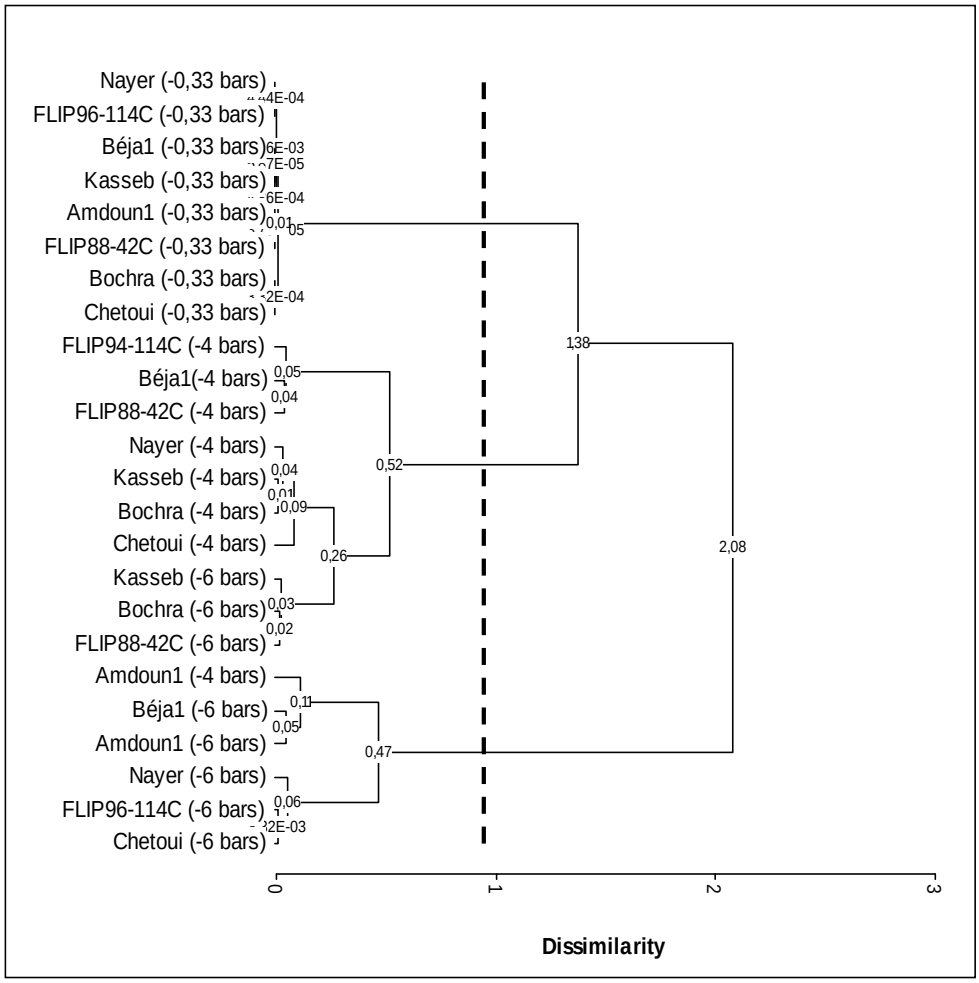

Figure 3. Chart of the hierarchical cluster analysis of the chickpea genotypes (*Cicer arietinum* L.) under different osmotic water pressures.

Table 5. Classes Barycentres resulting from the hierarchical cluster analysis of the chickpea genotypes (*Cicer arietinum* L.) according to germination parameters and soluble sugar and proline contents

Classes	GR (%)	GT(days)	GE (seeds/day)	SS (mg.g-1 of FM)	Pr (µmol.g-1 of FM)
Class 1	100,000	4,000	2,500	0,341	58,918
Class 2	96,667	4,516	2,245	4,494	157,687
Class 3	81,667	5,729	1,537	3,717	92,648

Abbs.: GR: Germination rate; GT: Germination Time; GE: Germination energy; SS: Soluble sugar content; Pr: Proline content.

The second group is at 11,93 Euclidean distance. It is composed of ten genotypes: $Beja_1$, Nayer, Kasseb, Bochra, FLIP94-114C, FLIP88-42C and Chetoui under the OWP -4 bars and Kasseb, Bochra and FLIP88-42C under the OWP -6 bars (Figure 3). Although the culture media are stressful, genotypes presented rapid germination, high germination rate and energy. They accumulated the highest levels of soluble sugars and proline (Table 5). Genotypes $Beja_1$, Nayer, Kasseb, Bochra, FLIP94-114C, FLIP88-42C and Chetoui appear osmotic water stress tolerant. The third group is located at 10,81 Euclidean distance. It consists of six genotypes: $Amdoun_1$ under the OWP -4 bars and $Beja_1$, $Amdoun_1$, Nayer, FLIP96-114C and Chetoui under the OWP -6 bars (Figure 3). Under -6 bars, these genotypes were sensitive to osmotic water stress. They took much more time to germinate with a reduced germination rate and germinative energy. They accumulated quite high soluble sugars and proline contents (table 5).

CONCLUSION

In vitro culture, Osmotic water stress, induced by the PEG_{8000} has negatively affected germination parameters of the kabuli chickpea genotypes and had favored osmoticum accumulation, in particular, soluble sugars and proline. A broad genotypic variability of the chickpea cultivars was detected toward the osmotic water stress. The OWP -8 bars proves very high and completely inhibited chickpea germination. Soluble sugars and proline accumulations are proportional to osmotic water stress. Hierarchical cluster analysis based on germination parameters and osmoticum accumulation revealed that under -6 bars, genotypes $Beja_1$, $Amdoun_1$, Nayer, FLIP96-114C and Chetoui are sensitive; whereas, Kasseb, Bochra and FLIP88-42C were tolerant to osmotic water stress. *In vitro culture*, selection of osmotic water stress tolerant chickpea genotypes, based on germination parameters and osmoticum accumulation is very informative. However, it could be one preliminary stage of rational screening consolidated by *in situ* researches.

REFERENCES

Ashraf M. and Harris P.J.C., (2004); Potential biochemical indicators of salinity tolerance in plants. Plant Sci. 166: 3-16.

Aspinall D. and Paleg L.G., (1981); Proline accumulation: Physiological aspects. In: Paleg, L.G., Aspinall, D. (eds.), Physiology and Biochemistry of Drought Resistance in Plants. Academic Press, New York, pp. 205-240.

Bajji M., Lutts S. et Kinet J.M., (2000) ; La résistance au stress hydrique chez le blé dur : Comparaison des comportements au niveau cellulaire et au niveau de la plante entière. In: Royo C. (ed.), Nachit M.M. (ed.), Di Fonzo N. (ed.), Araus J.L. (ed.). Durum wheat improvement in the Mediterranean region: Nouveaux défis. Zaragoza : CIHEAM-IAMZ; pages: 227-231.

Belhassen E., This D. et Monneveux P., (1995) ; L'adaptation génétique face aux conditions de sécheresse. Cahiers Agricultures; 4: 251-261;

Bellinger Y., Bensaoud A. and Larher F., (1991); Physiological significance of proline accumulation, a trait of use to breeding for stress tolerance. In: INRA (eds.) Physiology-Breeding of winter cereals for stressed Mediterranean Environments; Montpellier, France, 3-6 July 1989; Paris 1991 (Les colloques n° 55).

Bellinger Y., Bensaoud A. and Lahrer P., (1989); Physiological accumulation: a trait of use to breeding for stress tolerance. In: Acevedo, Conesa, Monneveux and Srivastava (eds): Physiology breeding of winter creates for stressed Mediterranean environments. Colloque n° 55, July 3-6, 1989, Montpellier, France, pp. 449-458.

Ben Salem M., (1993) ; Etude comparative de l'adaptation à la sécheresse du blé de l'orge et du triticale. In: INRA (eds) Tolérance à la sécheresse des céréales en zones méditerranéennes. Diversité Génétique et amélioration variétale. Les colloques de l'INRA, 64: 275-298.

Benlarabi M. et Monneveux P., (1988); Etude comparée du comportement en situation de déficit hydrique de deux variétés algériennes de blé dur (Triticum durum Desf.) adaptées à la sécheresse. C.R. Acad. Agric. France., 74 (5), pp. 73-83.

Blashfield R.K. and Aldenderfer M.S., (1988); The methods and problems of cluster analysis. In: J.R. Nesselroad (eds.), Handbook of multivariate experimental psychology, 2nd Ed. (447-473). New York: Plenum.

Blum A.B. and Ebercon A., (1981); Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sc. 21: 43-47.

Boubaker M. and Yamada T., (1995); Differential genotypic responses of string wheat early seedling growth to limited moisture conditions. Tropicultura; 13, 2: 50-53.

Buysse J. and Merckx R., (1993); An improved colorimetric method to quantify sugar continent of plant tissue. J. Exp. Bot. 44: 1627-1629.

Chinard F.P. (1952); Photometric estimation of proline and ornithine. J. Biol. Chem.199: 91-95.

Côme D. 1970. Les obstacles de la germination. Masson et Cie Ed., Paris ; 162p.

Crowe J.H., Hoekstra F.A., and Crowe L.M., (1992); Anhydrobiosis; Ann. Rev. Physiol. 54: 579-599.

Curtis J., Shearer G. and Kohl H., (2004); Bacteroïdes proline catabolism affects N2 fixation rate of drought-stressed soybeans. Plant Physiology, 136 (2): 3313-3318.

De Raissac M. (1992) ; Mécanismes d'adaptation à la sécheresse et maintien de la productivité des plantes cultivées. Agronomie Tropicale, 46 (1): 29-37.

Dirik H., (2000) ; Effets du stress hydrique osmotique sur la germination des graines chez les provenances du cèdre du Liban (Cedrus libani A. Rich) d'origine Turque. INRA, EDP, Sciences. Ann. For. Sci. 57: 361-367.

Djavanshir K. and Pourbeik H., (1976); Germination value- New formula. Silv. Genet. 25: 79-83.

Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A. and Smith F., (1956); Calorimetric method for determination of sugars and related substances. Analy. Chem. 28: 350-356.

Erskine W., Tufil M., Russell A., Tyagi M.C., Rahman M.M. and Saxzna M.C., (1994); Current and future Strategies in breeding lentil for resistance to biotic and abiotic stresses, Euphytica 73: 127-135,

Feutry A. et Bertrand D., (2003) ; Contrôle de la qualité des semences par vision artificielle. Recherche Innovation ; Les Rencontres du Végétal en Horticulture ; Semences et Paysage ; Semences et approches socio-économiques ; Résumés des communications; Les 20 et 21 novembre 2003 à l'Institut National d'Horticulture, Séminaire INRA - Angers.

Gaudillière J.P. et Barcelo M.O., (1990) ; Effets des facteurs hydriques et osmotiques sur la croissance des talles de blé. Agronomie; 10: 423-432.

Hooda A., Nandwal A.S., Kuhad M.S. and Dutta D., (1999); Plant water status and C, N and K distribution in potassium fertilized mung bean under drought and during recovery. In: Faroda, A.S., Joshi, N.L., Kathju, S., Karj, A. (eds.); Recent Advances in Management of Arid Ecosystem. Proceedings of a Symposium held in Jodhpur; India in March 1997. Arid Zone Research Association of India, pp. 207–214.

Hanson A.D., Nelson C.E. and Everson E.H., (1977); Evaluation of free proline accumulation as an index of drought resistance using two contrasting barley cultivars. Crop Sci.; 17: 720-726.

Ingram J. and Bartels D. (1996); The molecular basis of dehydration tolerance in plants; Annu. Rev. Plant Physiol. Plant Mol. Biol. 47: 377- 403.

Jaouadi, W., Hamrouni L., Souayeh N. et Khouja M.,L. (2010) ; Etude de la germination des graines d'Acacia tortilis sous différentes contraintes abiotiques ; Biotechnol. Agron. Soc. Environ. 14(4): 643-652.

Kameli A. and Losel G.M., (1995); Contribution of carbohydrates and other solutes to osmotic adjustment in wheat leaves under water stress, J. Plant Physiol. 145: 363-366.

Karamanos A., Drossopoulos J.B. and Aiavis C.A. (1983); Free proline accumulation during development of two wheat cultivars with water stress. J. Agric. Sci. 100: 429–439.

Kauss H. (1977); Biochemistry of regulation. In: NORTHCOTE (eds): International Review of Biochemistry, II, pp. 119-139.

Khouja M.L., Tej Yacoubi M., Souayah N. et Albouchi A., (2002) ; Influence d'un stress osmotique sur la germination de trois provenances de Tamarugo (Prosopis tamaruga Phil.). Annales de l'INGREF ; 5:153-163.

Kouakou T.H., Kone M., Kone D., Kouadio Y.J. et Zouzou M., (2008) ; Réponse physiologique au stade juvénile du génotype R405-2000 de cotonnier (Gossypium hirsutum L.) au déficit hydrique induit par le polyéthylène glycol; Sciences & Nature Vol. 5 N°1: 81 - 87.

Laouar M., Kies N., Abdellaoui K., Bennour A., Bettahar N., Kadi S., Bouzza L. et Abdelguerfi A., (2001) ; Effet du stress hydrique sur le comportement physiologique de dix populations de Medicago intertexta. In: CIHEAM-IAMZ (eds); Qualité de la luzerne et des medics pour la production animale. Options Méditerranéennes: Série A. Séminaires Méditerranéens; n° 45; Zaragoza (Spain); pp: 167-171.

Mefti M., Abdelguerfi A. et Chebouti A., (2001) ; Etude de la tolérance à la sécheresse chez quelques populations de Medicago truncatula (L.) Gaertn.. In: CIHEAM-IAMZ (eds); Qualité de la luzerne et des medics pour la production animale. Options Méditerranéennes: Série A. Séminaires Méditerranéens; n° 45; Zaragoza (Spain); pp: 173-176.

Milligan G.W., (1981); A review of Monte Carlo tests of cluster analysis. Multivariate Behavioral Research, 16: 379-407.

Morgan J.M. (1984); Osmoregulation and water stress in higher plants. Annu. Rev. Plant Physiol. 35: 299–319.

Muhammad A. and Iram A., (2005); Drought stress induced changes in some organic substances in nodules and other plant parts of two potential legumes differing in salt tolerance; Flora; 200: 535–546;

Munoz F.J., Labrador E. and Dopico B., (1998); A cDNA encoding a proline-rich protein from Cicer arietinum L. Changes in expression during development and abiotic stresses. Physiol. Plant, 102: 582–590.

Newton R.J., Bhaskaran S., Juryear J.D. and Smith R.H., (1986); Physiological changes in cultured sorghum cells in response to induced water stress. Soluble carbohydrates and organic acids. Plant Physiology, 36: 626-629.

Pedersen A., Feldner H. and Rosendahl L., (1996); Effect of proline on nitrogenous activity in symbiosomes from root nodules of soybean (Glycine max L.) subjected to drought stress. Journal of experimental Biology, 47: 1533-1539.

Radhouane L., (2011) ; Accumulations métaboliques en présence de contraintes hydriques chez le mil (Pennisetum glaucum L. R. Br.); Revue des Régions Arides n° 25 (1/2011):15-34

Sané D., Ould Kneyta M. and Diouf D., (2005); Growth and development of date palm (Phoenix dactylefera L.) seeding under drought and salinity stresses. African Journal of Biotechnology. vol.4; 9: 966-972.

Santarius K.A., (1973); The protective effect of sugars on chloroplast membranes during temperature and water stress and its relationship to frost, desiccation and heat resistance. Planta; 113: 23-46.

Savitskaya N.N., (1976); On the physiological role of proline in plants. Biol. Nauki (Moscow) 19: 49–61.

Singh T.N., Paleg L.G. and Aspinall D., (1973); Nitrogen metabolism and growth in barley plant during water stress. Aust. J. Biol. Sci., 26: 45-56.

Slim, N., Sifi B. et Triki S., (2006); Criblage de variétés de pois chiche (Cicer arietinum L.) pour la résistance au stress hydrique. In: Séminaire International (eds) Gestion des Ressources et Applications Biotechnologiques en Aridoculture et Cultures Sahariennes: Perspectives pour la valorisation des potentialités du Sahara.; Djerba, du 25 au 28 Décembre 2006.

Sokal R.R. and Sneath P.H.A., (1963); Principles of Numerical Taxonomy. Freeman and co., San Francisco, London; 359p.

Tahri, E.H., Belabed A., Sadki K., (1998) ; Effet d'un stress osmotique sur l'accumulation de proline, de chlorophylle et des ARNm codant pour la glutamine synthétase chez trois variétés de blé dur (Triticum durum Desf). Bull. Inst. Sci., Rabat, 21: 81-87.

Turner N.C., (1986); Adaptation to water deficits: A changing perspective. Aust. J. Plant Physiol. 13: 175-190.

Vachon M., Beaulieu-Prévost D., Ouellette A. et Achille M., (2005); Analyse de classification hiérarchique et qualité de vie; Tutorials in Quantitative Methods for Psychology Vol. 1 ; 1: 25-30.

Venekamp J.H., Lampe J.E.M. and Koot J.E.M., (1989); Organic acids as sources for drought-induced praline synthesis in field bean plants, Vicia faba L. Journal Plan Physiology, 133: 654-659.

Zerrad W., Hillali S., Mataoui B.S. El Antri S. Et Hmyene A., (2006); Etude comparative des mécanismes biochimiques et moléculaires de résistance au stress hydrique de deux variétés de blé dur. Congrès International de Biochimie. Agadir, 09-12 Mai 2006; Biochimie, Substances Naturelles et Environnement; 371 - 376.

Zhang B. and Archbold D.D., (1993); Solute accumulation in leaves of a Fragaria chiloensis and Fragaria viginiana selection responds to water deficit stress. J. AN. Soc. Hort. Sci. 118: 280-285.

Zhang J., Nguyen H.T. and Blum A., (1999); Genetic analysis of osmotic adjustment in crop plants. J. Exp. Bot., 50: 291-302.

Zid E. et Grignon C., (1991); Les tests de sélection précoce pour la résistance des plantes aux stress. Cas des stress salin et hydrique; L'amélioration des plantes pour l'adaptation aux milieux arides. Ed. AUPELF-UREF. John Libbey Eurotext. Paris; pp: 91-108.