IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
ISSN: 2319-2380, ISBN: 2319-2372. Volume 2, Issue 1 (Jan. - Feb. 2013), PP 50-56
www.iosrjournals.org

Effect of Drought on Growth and Chlorophyll Content in Three Vetch Species

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***Abstract*:** Plants in the nature are constantly exposed to biotic and abiotic stresses. Among these stresses, the drought is the mainly important factor unfavorable to the growth and the development of plants, and it is considered as a threat in the sustainable agriculture especially in dry regions. The aim of this work is to study the effect of 4 water regimes [100% (control) - 80 - 60 and 40% of the field capacity (FC)] on the dimensional and weight growth and on the chlorophyll content in three vetch species (*Vicia narbonensis*, *V. sativa* and *V. villosa*). The experiment was carried out under greenhouse in a randomized complete block design with four replicates. The sowing was realized by one pre-germinating seed per pot. The water stress was imposed at the two leaves stage. The parameters were determined after 71 days of drought. Results showed that height, leaf area and dry matter of shoots and roots decreased significantly with the water stress intensities on the three vetch species. However, *V. villosa* was the most sensitive one at this stage of growth, compared to Narbonne vetch. The biomass reduction was in concomitance with an important decrease in chlorophyll content which reached 44% and 20% respectively for *V. villosa* and *V. narbonensis.*
Keywords: Chlorophyll, growth parameters, vetch, water stress.

I. Introduction

Vetch (*Vicia ssp.*) is a leguminous, annual, and commonly used forage in the Mediterranean basin, in pure or in mixture with winter cereal (oat, triticale) under rainfed conditions. The vetch improves the soil fertility and constitutes a good cultural cropping for the wheat (Dalias, 2012). In Tunisia, deficiency in forage and pasture productions is due mainly to the reduction in feed productivity which have been attributed to a decrease in rainfall and consequently an increase in drought frequencies (Chakroun et al., 2011). The major consequences of the climate change for the Mediterranean agriculture is the increase of the risks of drought (Gaufichon et al., 2010; De Oliveira et al., 2012). Indeed, according to Nasr et al., (2009) climate change projections for Tunisia predict, in horizon 2050, a decrease in rainfall around 10 to 30% from north to south. The drought is widely recognized as the first limiting factor to the agricultural production (Chaves and Oliveira, 2004; Anselm and Taofeeq, 2010.). In these conditions, only the adapted plants can survive. That is the major challenge of the research programs to develop resistant or tolerant varieties to water stress (Almeselmani et al., 2012). Indeed, Sharp et al., (2004) and Tardieu, (2005) signaled that understanding the genetic and the biochemical mechanisms which control drought tolerance is a central question in plant biology and the development of drought-tolerant lines will become increasingly important.

The responses of plant to water deficit are seen through a morphological modifications with a decrease in dry matter and height (Zubaer et al., 2007 ; Oktem, 2008 ; Praba et al., 2009 ; Nagarajan and Nagarajan, 2010 ; Anjum et al., 2011 ; Shooshtarian et al., 2011) ; with a reduction of the leaf expansion which has a consequence in the water losses by stomata and cuticular transpiration (Burghardt and Riederer, 2003 ; Hufstetler et al., 2007 ; Damour et al., 2010 ; Andivia et al., 2012) , with anatomical change (Denden et al., 2005 ; Saadu et al., 2009 ; Abdel et al., 2011a) and biochemical such as the accumulation of organic compounds (osmoprotectant) like proline, glycine betaïne whose protect proteins and membranes against damage by high concentrations of inorganic ions (Rathinasabapathi, 2000 ; Garg *et al.*, 2001 ; Abebe *et al.*, 2003 ; Akinci and Losel, 2009 ; Akinci and Losel, 2010).

A severe water stress provoked a functional damage and premature senescence of the leaves (Chaves *et al.*, 2002). The main consequences of a water stress are the decrease in the growth caused by a reduced of the photosynthesis (Ashraf et al., 2001). The photosynthesis is the process by which plants combine the water and the CO_2 thanks to the energy of the light to synthesize carbohydrates (Brodribb et Holbrook, 2003 ; Dogan *et al.*, 2007 ; Petropoulos *et al.*, 2008).

Tardieu, (2005) resumed the strategies for developing plants able to grow in dry environment scenarios face essentially the same type of choices. Plants can consist in (*i*) escaping the water deficit, for example by a short crop cycle which allows plants to finish their cycle before severe water stress sets in, (*ii*) avoiding the water deficit, for instance by reducing transpiration or increasing water uptake, (*iii*) maintaining growth under water deficit, (*iv*) resisting severe deficit through survival mechanisms. The objective of the present work was to investigate the growth parameters and chlorophyll content in presence or absence of water stress conditions in three vetch species in order to select drought resistant or tolerant genotypes.

II. Materials and methods

Plant material and growth conditions

Four water regimes [100% (control) - 80 - 60 and 40% of the field capacity (FC)] were applied on three vetch species: *Vicia sativa*, *V. villosa* and *V. narbonensis*. The experiment was carried out under greenhouse in a randomized complete block design with four replicates (3 species x 4 treatments x 4 repetitions = 48 pots). The sowing was realized by one pre-germinating seed per pot of 16cm diameter. The water stress was imposed at the two leaves stage. The parameters were determined after 71 days of drought. The quantity of water requirement for each treatment was determined by mini-lysimètres.

Measurement of parameters

The growth parameters (plant height, leaf area, dry matter and roots/shoots ratio) were determined after 71 days of water stress. Dry matter of different organs (leaves, stems and roots) was obtained by desiccation at 50°C. Leaf area was measured for each treatment by photocopying the leaves, cutting leaf footprints, weighing them and then relating this weight to that of a paper of known weight and surface (Mezni et al., 1999 ; Garcia et al., 2002).

The extraction of chlorophyll was determinate in fourth leaf rank per plant and per replicate. Fresh tissue was sampled from the youngest fully expanded leaf (1g), extracted with 80% acetone and read using a UV/Visible Spectrophotometer at λ = 663 and λ = 645 nm wavelengths. Chlorophyll concentrations (mg.g^{-1} DM) were calculated using the formula given by Mackiney (1941): $\boldsymbol{Chl = 20{,}2\ OD_{\lambda 645} + 8{,}02\ OD_{\lambda 663}}$; Were **OD** = optical density.

Statistical analysis

The variance analysis was realized by the software SAS (version 8.0) via ANOVA procedure. The comparison between species and treatments was made with the Duncan test. Confidence intervals were calculated to the threshold of 95% probability.

III. Results

Leaf area

Leaves constitute the most active organ of the plant. It is the seat of all the biochemical activities. The Figure 1 showed that the leaf area undergoes a significant decrease with the drought intensity for the three vetch species. The severe reduction was observed in *V. villosa* under the stressful treatment (40% FC). Compared to *villosa* and *sativa* species, *V. narbonensis* was the least affected by the water stress.

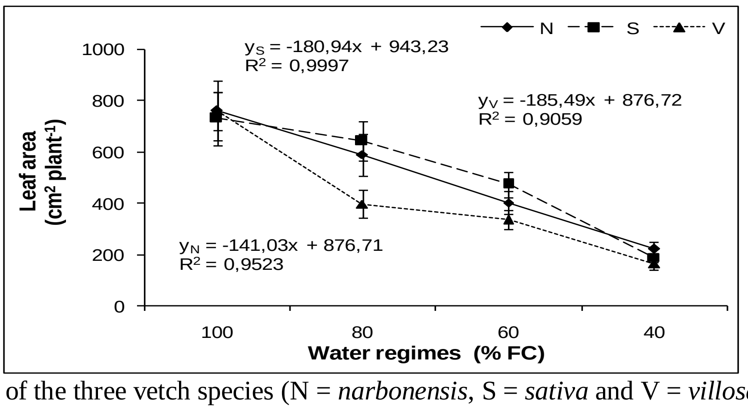

Figure 1. Leaf area of the three vetch species (N = *narbonensis*, S = *sativa* and V = *villosa*) under four water regimes (100 - 80 - 60 and 40%FC). Each value represented the mean of 4 replicates. Confidence intervals were calculated at α = 95% probability level.

Plant height

Compared to the other species, *V. narbonensis* had the more important height in absence and presence of the drought. In presence of moderate stress (80%FC), the height of *V. sativa* was not significant different with the control (fig. 2). At the stressful treatment (40%FC), there is no significant difference for the height between the three species.

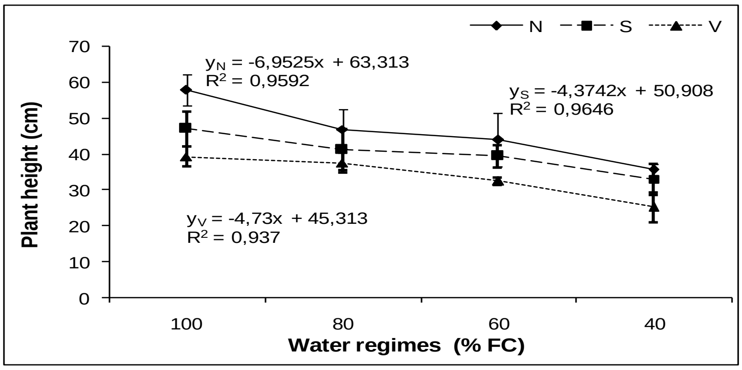

Figure 2. Height of the three vetch species (N = *narbonensis*, S = *sativa* and V = *villosa*) under four water regimes (100 - 80 - 60 and 40%FC). Each value represented the mean of 4 replicates. Confidence intervals were calculated at α = 95% probability level.

Shoot Dry Matter (DM)

The dry matter is the resultant of the growth of the height and the carbohydrates accumulation via photosynthesis. The DM was severely reduced by the increase of the water stress intensity for the three vetch species. Shoot DM of *V. narbonensis* was significantly important at the stressful treatments (60 and 40%FC), where the DR reduction was 53% under 40%FC (fig. 3), compared to 70% of reduction, respectively for *villosa* and *sativa* species, at the same treatment (40%FC).

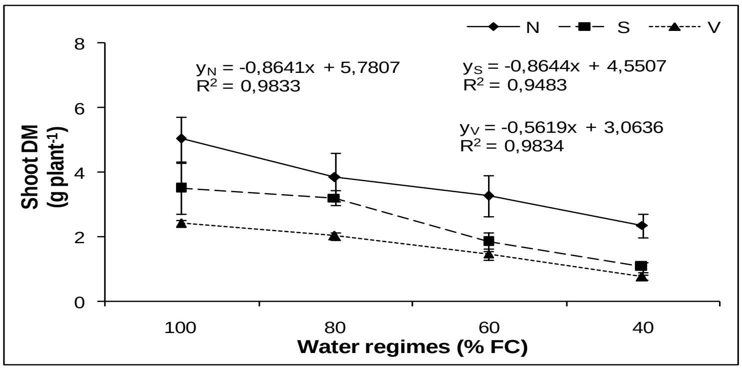

Figure 3. Shoot DM of the three vetch species (N = *narbonensis*, S = *sativa* and V = *villosa*) under four water regimes (100 - 80 - 60 and 40%FC). Each value represented the mean of 4 replicates. Confidence intervals were calculated at α = 95% probability level.

Root Dry Matter

The root dry matter of *Narbonne* vetch was not affected by the increase of the water stress in pots. While *V. villosa* root DM undergoes a linear reduction with the water stress intensity (fig. 4). For *V. sativa*, the root dry matter was severely reduced by the intensity of the drought (60 and 40%FC). At the stressful treatment (40%FC), there is no significant difference between *sativa* and *villosa* species.

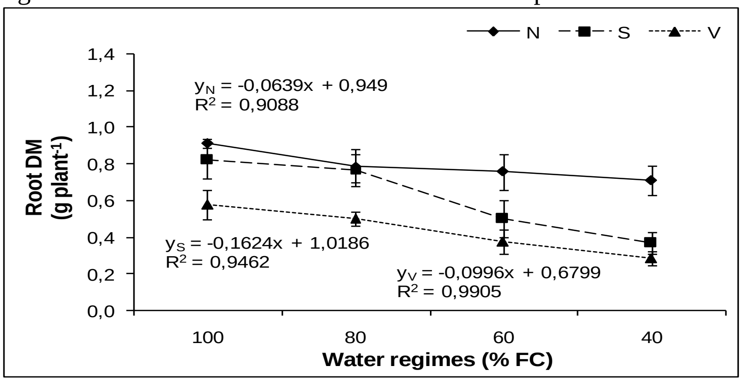

Figure 4. Root DM of the three vetch species (N = *narbonensis*, S = *sativa* and V = *villosa*) under four water regimes (100 - 80 - 60 and 40%FC). Each value represented the mean of 4 replicates. Confidence intervals were calculated at α = 95% probability level.

Root/shoot ratio

The root/shoot ratio of the three vetch species increased with the increase of the intensity of the water stress in pots (fig. 5). This indicated that root DM was less affected by the drought, compared to the shoot DM. Vetch plants developed more roots to investigate a supply of deep horizon of soil.

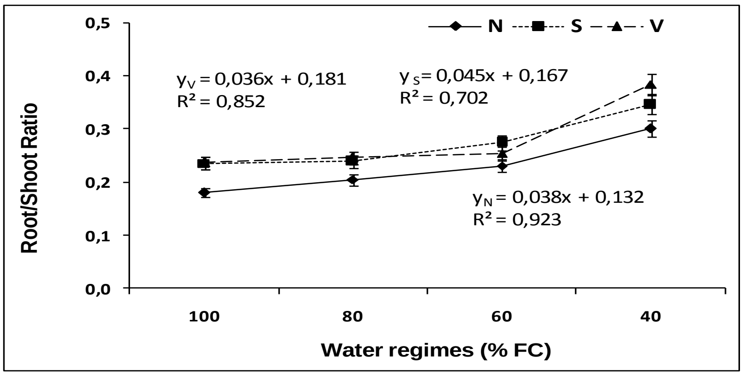

Figure 5. Root/Shoot ratio of the three vetch species (N = *narbonensis*, S = *sativa* and V = *villosa*) under four water regimes (100 - 80 - 60 and 40%FC). Each value represented the mean of 4 replicates. Confidence intervals were calculated at α = 95% probability level.

Chlorophyll content

V. sativa has the high level of the chlorophyll content under all treatments (Fig. 6). The decrease in chlorophyll content for *narbonensis* and *sativa* species was not significantly different between the control and the water stressful treatment, while *V. villosa* pigment decrease significantly with the rise of the drought intensity and reached a decrease of 44% under the stressful treatment (40%FC).

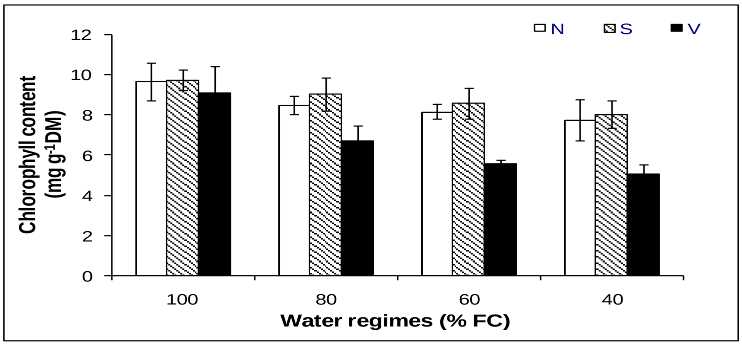

Figure 6. Chlorophyll content of the three vetch species (N = *narbonensis*, S = *sativa* and V = *villosa*) under four water regimes (100 - 80 - 60 and 40%FC). Each value represented the mean of 4 replicates. Confidence intervals were calculated at α = 95% probability level.

IV. Discussion

In the present study, we investigate the dynamics of the drought through a certain growth parameters of three vetch species. Indeed, the morphological modifications observed on the vetch species under water stress were used as a criterion to tolerate the water deficiency. The most effect of water stress on vetch was the reduction of leaf area, which has a direct consequence in limitation of the water losses by stomatal and cuticular transpiration as mentioned by Noitsakis et al., (1991) and Blum, (2005).

The decrease in vetch LA with the rise of the intensity of water stress confirm the result found by Tardieu, (2005) which he indicated that under drought, if the roots cannot uptake water, the plant slowed down its transpiration by closing stomata and limiting foliar growth. The closure of stomata and the decrease in the foliar growth allowed a homeostasis of the water foliar state, but reduced also the photosynthesis. Prada et al., (2009) observed in two rice cultivars and three wheat varieties subjected to water stress a decrease in the leaf elongation and membrane stability. Indeed, in wheat seedlings subjected to a mild water stress (Ψw=-0.3 MPa), Schuppler et al., (1998) found that the leaf-elongation rate was reduced by one-half and the mitotic activity of mesophyll cells was reduced to 42% of well-watered controls within 1 day. The leaf area reduction is considered as a criterion of adaptation to the drought (Masinde et al., 2006). However, the decrease of the leaf area in the presence of the water deficit was resulted in a reduction of the dry matter accumulation (Martinielli and Da Silva, 2011).

Indeed, the water losses on the three vetches pulled a down in guard cell and the closure of stomata (Chaves et al., 2003 ; Sanchez-Blanco *et al*., 2009). According to Flexa and Medrano, (2002) the inhibition of net photosynthesis under water stress may result in part from lower diffusion of CO_2 across leaf mesophyll. Also, an important reduction in the vetch plant height was observed, resulting from a simultaneously inhibition of the cellular division and from a slowing down in the cells elongation of the enter-nodes (Granier et al., 2000 : Costa e Silva et al., 2004 ; Läuchli and Grattan, 2007 ; Martin et al., 2008). Besides, the water deficit reduced the accumulation of the dry matter of the three vetch species, as mentioned by Akmal et al., 2010. This phenomenon was common to most of the cultivated species such as: ***cowpea*** (Dadson et al., 2005 ; Lobato et al., 2008 ; Ahmed and Suliman, 2010) ; ***Rice*** (Zubaer et al., 2007) ; ***Soya*** (Hufstetler et al., 2007 ; Martins et al., 2008 ; Demirtas et al., 2010 ; Makbul et al., 2011) ; ***alfalfa*** (Loussaief, 2002 ; Petcu et al., 2009 ; Slama et al., 2011) ; ***wheat*** (Petcu, 2005) ; ***vetch*** (Abdel and Al-Rawi, 2011b ; Bilgili et al., 2011 ; Çöçü and Uzun, 2011) and ***corn*** (Earl and Davis, 2003).

Our results showed an increase of the root/shoot ratio as was mentioned by Bingcheng *et al.* (2007) and Slama *et al.* (2008). This indicated that water stress has entailed a severe decrease in the biomass air part than the root biomass. According to Slama et al., (2006), root/shoot DM ratio is a criterion of plant adaptation to the drought.

This indicated that the plant, in response to water deficit, favors the translocation of assimilats towards roots to investigate a more important of soil volume. Indeed, Achten et al., (2010) indicated in *Jatropha curcas*, subjected to a severe water stress, that the plant falls its leaves and accumulates more dry matter in roots. Silva et al., (2010) indicated in *Erythrina velutina* submitted to drought, that the maintenance of turgor pressure seems to be more associated with a reduction in the growth ratio than a reduction in leaf water potential. For Davies (2006), the reduce in soil water availability decreased water uptake by plants and also restricted nutrient uptake by roots and transport to the shoots what in turn reduced the growth.

Besides, several authors showed that the water stress entailed a decrease of the quantity of chlorophyll in leaves (Fu and Huang, 2001 ; Zhang et al., 2007 ; Sanchez-Blanco *et al*., 2004 ; El Tayeb, 2006 ; Abdalla and El-Khoshiban, 2007 ; Terzi et al., 2010). According to Keyvan (2010), the decrease of the chlorophyll content, in conditions of water deficit, is considered as a non stomatic limiting factor.

V. Conclusion

The various growth parameters showed that in absence of a water stress, *V. narbonensis* was characterized with a high LA, height and DM, compared to the other species. In conditions of water stress, *V. narbonensis* was also the most tolerant, followed by *V. sativa*. *V. villosa* was the most affected by water deficit. However, the three vetch species develop their root organs compared to their shoot one to investigate deeper soil horizons and they limit their water losses by a severe reduction in leaf area.

Acknowledgements

This study was performed within the support of the Faculty of Tunis Science and the Institute of Agronomic Research of Tunis.

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