

## Effects of the defoliation height on the growth, mineral uptake and soluble carbohydrate contents in berseem clover (*Trifolium alexandrinum* L.) var. *Khadhraoui*

Mezni\*<sup>1</sup> M., Haffani<sup>1</sup> S., Khamassi<sup>2</sup> N., Albouchi<sup>3</sup> A.

<sup>1</sup>Université de Carthage. Laboratoire des Productions Animales et Fourragères. INRAT, Rue Hédi Karray 2049, Ariana, Tunisie.

<sup>2</sup>Laboratoire de Physiologie Végétale, Faculté des Sciences de Tunis. Campus Universitaire, 1060, le Belvédère, Tunis, Tunisie.

<sup>3</sup>Unité d'Agro-Sylvo-Pastoralisme. INRGREF, BP 10, 2080, Ariana, Tunisie.

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**Abstract:** In a pot experiment the responses of berseem clover (*Trifolium alexandrinum* L.) var. *Khadhraoui* to the defoliation heights (2.5 -5 -7.5 and 10cm) were evaluated in terms of shoot and root growth,  $K^+$ ,  $Ca^{2+}$  and P inorganic uptake and soluble carbohydrates. Plants were grown in green house under natural light and temperature. The experimental was conducted using a complete randomized block design with 4 repetitions. Berseem was defoliated when plants achieved 50cm of height. Results showed that the cumulative dry material varies with the defoliation height. In the sixth harvest treatment, DM under the 5cm cutting height was significantly higher than in the other treatments. The leaf area (LA) per plant showed a depressive effect of the extreme defoliations: shorter (2.5cm) and higher (10cm), while the cutting heights at 5 and 7.5cm present a LA appreciably similar. The specific leaf area (SLA) decreases with the increase in the defoliation heights and a significant difference is observed between plants defoliated at 2.5 and 5cm, on one hand and those harvested at 7.5 and 10cm, on the other hand. The Stem/Leave ratio is a good index for the appreciation of fodder quality. Indeed, the defoliation heights at 2.5 and 5cm have a fodder quality better than those defoliated at 7.5 and 10cm. Compared to the other treatments, the Shoot/Root ratio under the defoliation height of 5cm was the lowest.  $K^+$  content in roots, stems and leaves decreased with the increase in the defoliation heights. Calcium content was not affected by the defoliation heights. The phosphorus decreased significantly with the cutting heights and the time of the harvest in the three organs of the berseem *Khadhraoui*. The soluble sugar contents in roots, stems and leaves of the berseem showed a severe decline in the root system. Results suggest that soluble carbohydrate reserves in roots can support plants regrowth after defoliation.

**Keywords:** Berseem, Calcium, Defoliation height, Growth parameters, Phosphorus, Potassium, Soluble carbohydrates.

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### I. Introduction

In Tunisia, the development of the livestock, a priority sector of the agricultural production, largely relies on the availability of feed resources mainly cultivated forage. The most used fodder belongs to the two botanical families: Grasses and Legumes which are selected to support successive cuttings for dry matter production throughout their vegetative cycle (Tessama et al., 2010). The berseem is widely cultivated in Tunisia. Berseem clover is an annual forage legume, commonly subjected to repeated cycles of defoliation and regrowth (Iannucci and Martiniello, 2000). This specie is an important legume due to its high content of proteins (De Santis et al., 2004), and because of its capacity to improve soil fertility by the organic matter and the symbiotic fixation of the atmospheric nitrogen (Mezni et Sifi, 1995). The berseem clover tolerates the low temperatures of the winter and vegetates after each harvest. The berseem is sown either alone or in mixture with ryegrass (*Lolium multiflorum* L.). The use of the mixture of berseem-ryegrass increased the total herbage yield, produced forage with high proteins and at lower cost (Mezni et al., 2000 ; Peyraud et al., 2009 ; Giambalvo et al., 2011a). Plant cutting or defoliation is a mechanical stress which deprives the plant of its system photosynthetic (leaves) and can compromise its growth and development. The regrowth will depend largely on the speed with which the defoliated plant can replace its foliar system. The cutting height is a very important parameter which influenced the plant yield, the quality of the harvested forage and the plant regeneration speed and the sustainability of the culture (Vinther, 2006 ; Baatar, 2008 ; El-Morsy, 2009 ; Wijitphan et al., 2009 ; Da Silveira et al., 2010 ; Chen et al., 2012; Onyeonagu and Ugwuanyi, 2012).

In berseem, after defoliation, two types of stems ensure the regrowth. Indeed, as reported by Dufour et al., (1989), (i) stems appeared on the basis remainder of stems left after cutting and (ii) stems directly regenerated from the crown. In a study on the effect of the intensity and the cutting height on Lucerne, Leach

(1968) showed that the severe defoliation highlights the role of leaves left after the cutting (residual leaf area), in the regeneration of the plant shoots.

Undersander and Pinkerton, (1989) and Dhont et al., (2006) indicated that the cutting management of alfalfa determines both the quality of the harvested forage and the health and stand life of the crop. In Berseem clover, Giambalvo et al., (2011b) noted that defoliation management of the forage crops affects endogenous reserves in the crowns and the taproots, the residual leaf area, and, consequently, the regrowth and biomass yield. Indeed, the regrowth of forage legumes after each harvest depends on energy reserves stored in the taproots and the crowns of the plants (Berthier et al., 2009). In practice, farmers have to take into account three points: (i) maximize yield, (ii) maintaining high quality forages and (iii) plant longevity.

The regrowth after the defoliation depends initially on the development of the photosynthetic leaves. The photosynthesis decreases severely, after defoliation or grazing (Schnyder and De Visser, 1999; Avicé et al., 2001). So, after the defoliation, the capacity of photosynthesis decreases and the regeneration will depend on the mobilization of the organic reserves (C and N) to the defoliated plants (Johansson, 1993; Goulas et al., 2002; Amiard et al., 2003; Meuriot et al., 2004 ; Lestienne et al., 2006; Lee et al., 2010). Several authors highlighted the importance of the carbohydrates and proteins reserves in the regrowth of plants after the defoliation (Gordon et al., 1999 ; Morvan-Bertrand et al., 1999 ; Simon et al., 2004). The objective of this work was to analyze the physiological and agronomical response of the berseem clover var. *Khadhraoui* to four cutting heights in terms of an optimal dry matter production with high protein contents.

## II. Materials and methods

### *Plant material and growth conditions*

The choice of the local berseem clover (*Trifolium alexandrinum*) var. *khadhraoui* in this study was its wide use in the irrigated area for the feeding of dairy cattle. Under irrigation, this variety can give annually 6 to 8 harvests. Four cutting height levels (2.5 -5 -7.5 and 10cm) up the crown were applied on the *khadhraoui* variety. The experiment was carried out under greenhouse in a randomized complete block design with four replicates (1 variety x 4 treatments x 4 replications = 16 pots). The sowing was carried using thirteen pre-germinating seeds per pot. Cement pots contained 22kg of basic soil (pH=8.2), rich in active chalk (7.5%), with a humidity fluctuating between: 7.8% at wilting point and 17.5% at field capacity (table 1). Pots were irrigated daily with tap water and continuously drained.

**Table 1.** Chemical and physical soil properties at the experimental site.

Parameters	Values
Humidity at field capacity (%)	17.5
Humidity at wilting point (%)	7.8
Clay (%)	25.0
Loam (%)	39.5
Sand (%)	44.0
pH	8.3
Electric conductivity (mmhos/cm)	3.5
Total chalk (%)	14.5
Active chalk (%)	7.5
Organic matter (%)	0.73
Carbon	0.4
Saturation ml/100g	25.0

### *Measurement of parameters*

The growth parameters (leaf area, shoots and roots dry matter) were determined when plant height achieved 50cm. Before each harvest, one plant per pot was carefully pulled up and then separated into roots and shoots. Dry matter of different organs (leaves, stems and roots) was obtained by desiccation at 70°C. The leaf area (LA) was calculated by the method of weighting, on leaves completely developed. Leaves are quickly photocopied and their imprints carefully are cut and weighed. The LA was then deducted, with the weight of a known surface. The LA per plant was obtained by multiplying the LA average by the corresponding leaves number (Garcia et al., 2002). The specific leaf area (SLA) designed the ratio of leaf area to leaf dry mass and expressed in  $\text{cm}^2 \cdot \text{mg}^{-1}$  leaves DM (Garnier et al., 2001).

Mineral analysis ( $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $(\text{PO}_4)^{3-}$ ) and carbohydrates were carried out on lyophilized samples, harvested when plants reached 50cm of height. Extraction of cations was achieved using the nitric technique (nitric acid to 0.5%). The  $\text{K}^+$ ,  $\text{Ca}^{++}$ , were measured by a flame photometer (Eppendorf photometer). The phosphate ion was measured by photolorimetry at a wavelength of 420nm. Carbohydrates were extracted with boiling alcohol at 70°. Total soluble sugar was determinate using a colorimetric method at 470 nm wavelengths.

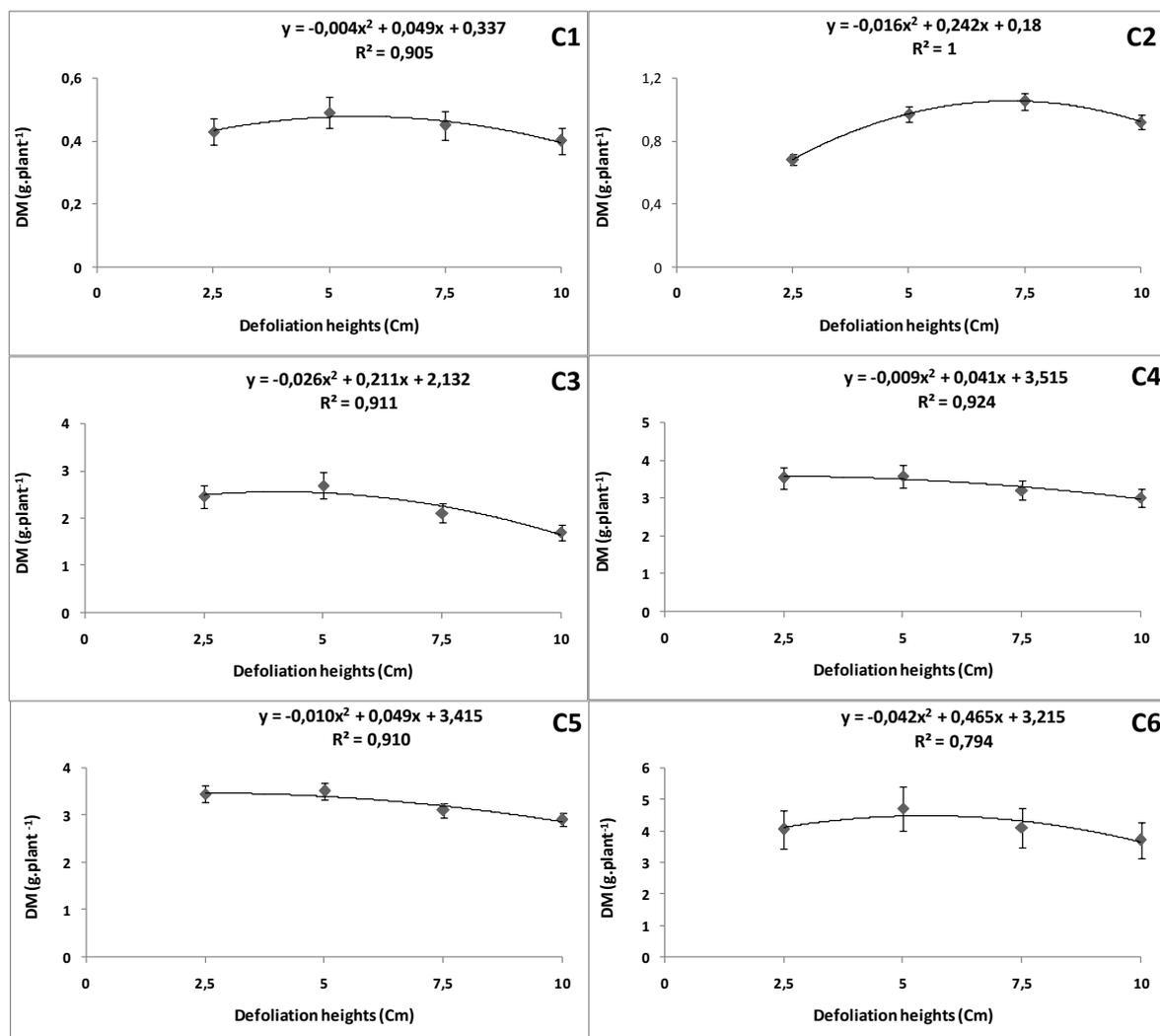
### Statistical analysis

Confidence intervals were calculated to the threshold of 95% probability. General Linear Models of SAS was used to explain the degree of significance of each factor and of the interactions between different factors. The Duncan test was used to compare treatment means for all studied parameters.

## III. Results

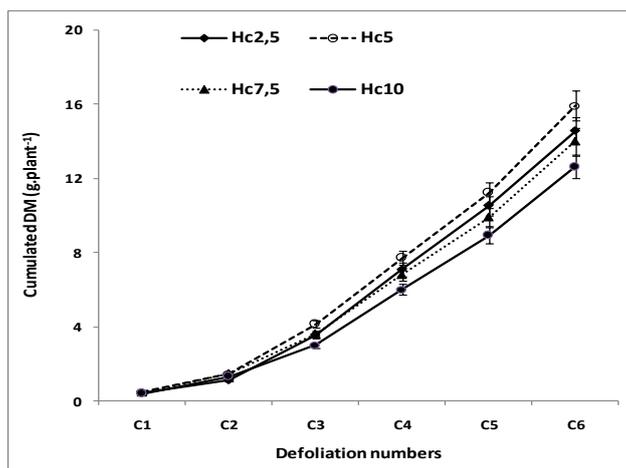
### Shoot dry matter (DM)

Figure 1 represents the variation of the shoot dry matter per defoliation according to the cutting heights. Results showed that dry matter varies with the increase of the defoliation height, according to a polynomial function of type:  $y = ax^2 + bx + c$ , Where  $y$  represents the DM (g) and  $x$  the defoliation height (cm). For the six harvests, the DM was the highest under the defoliation height of 5cm (except the C2).



**Figure 1.** Shoot DM, under defoliation heights (2.5 - 5 - 7.5 and 10cm) on the berseem *Khadhraoui*. Each point is the mean of 4 individual values. Intervals of confidence were calculated at  $\alpha = 95\%$ .

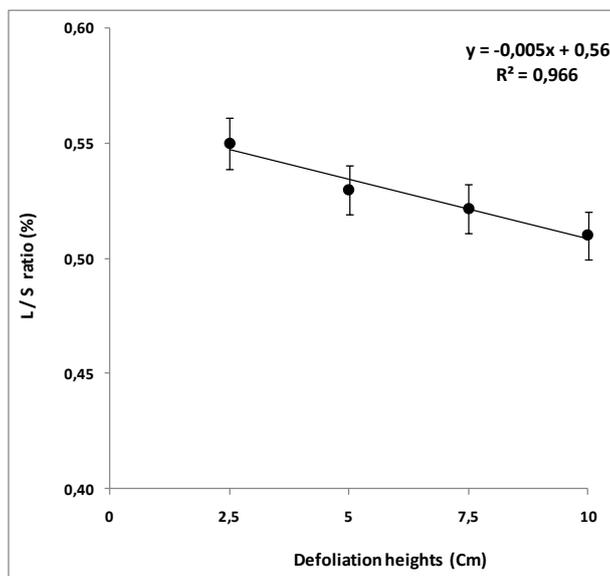
Figure 2 showed that cumulated DM was significantly higher under the defoliation height of 5cm, compared to 10cm treatment starting from the third harvest; while DM was significantly different with 7.5cm treatment only from the fourth harvest (C4). There was no significant difference between defoliation heights of 2.5 and 7.5cm, and this is for all the harvests.



**Figure 2.** Variation of cumulated shoot DM, with the defoliation numbers on the berseem *Khadhraoui*. Each point is the mean of 4 individual values. Intervals of confidence were calculated at  $\alpha = 95\%$ .

**Leaf/stem (L/S) ratio.**

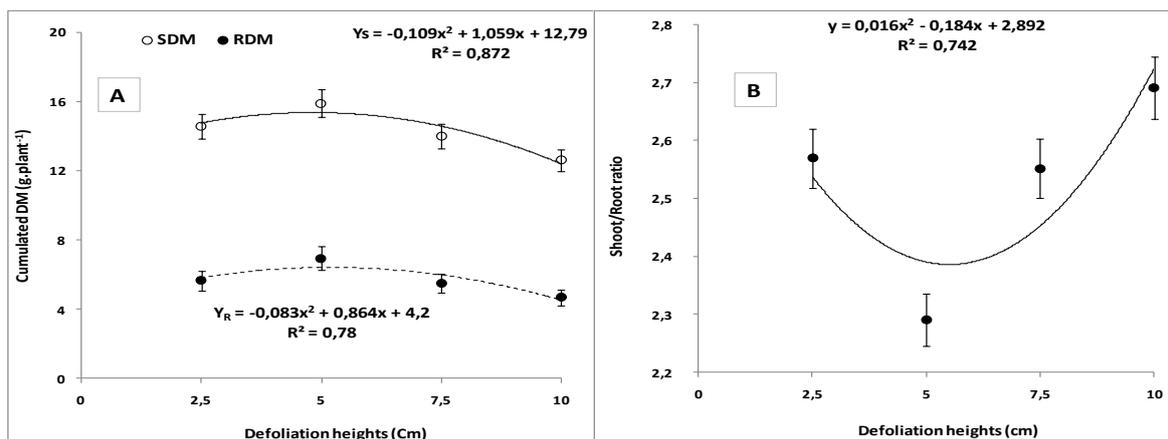
The leaf/stem ratio is a very important agronomic criterion which expresses the fodder quality of the plant and shows the energy value of the specie (Lemaire et al., 1994). Figure 3 represents the variation of the L/S ratio in relation to the defoliation heights. Results show that the L/S ratio decreases linearly with the increase in the defoliation heights. The decrease in L/S ratio can be explained by the senescence of the old leaves, left below the defoliation at 7.5 and 10cm height. While the stem dry matter is constituted by the stems neoformed but also by a part of the rests of the previous cut which exceeded the defoliation height recommended.



**Figure 3.** Leaf/stem (L/S) ratio under defoliation heights (2.5 - 5 - 7.5 and 10cm) on the berseem *Khadhraoui*. Each point represents the mean of 4 individual values. Intervals of confidence were calculated at  $\alpha = 95\%$ .

**Cumulated shoot and root dry matter and Shoot to Root (S/R) ratio.**

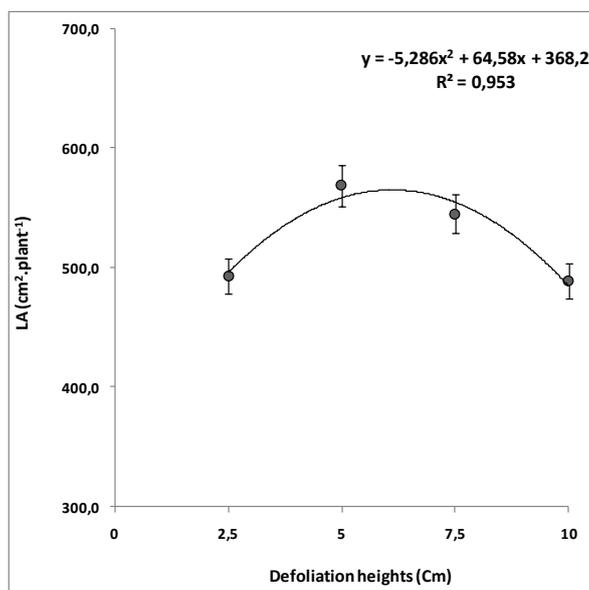
To explain Shoot/Root ratio (figure 4B), we look at the variation of the dry matter of shoots and roots (figure 4A). The dry matter, in shoot and root, is affected by the defoliation height in the same direction. The DM under the defoliation height of 5cm was the highest. The S/R ratio varies with the defoliation heights according to a hyperbolic curve:  $y=ax^2+bx+c$ ; where  $y$  represents the (S/R) ratio and  $x$  the defoliation height (cm). It appeared that the S/R ratio under the defoliation height of 5cm is the lowest ( $P<0.05$ ), compared to the other treatments. This indicates that the root DM increases under the defoliation height of 5cm faster than that of the shoot DM.



**Figure 4.** Shoot (SDM) and Root (RDM) cumulated DM (A) and Shoot/Root ratio (B) under defoliation heights (2.5 - 5 - 7.5 and 10cm) on the berseem *Khadhraoui*. Each point is the mean of 4 individual values. Intervals of confidence were calculated at  $\alpha = 95\%$ .

**Leaf area (LA)**

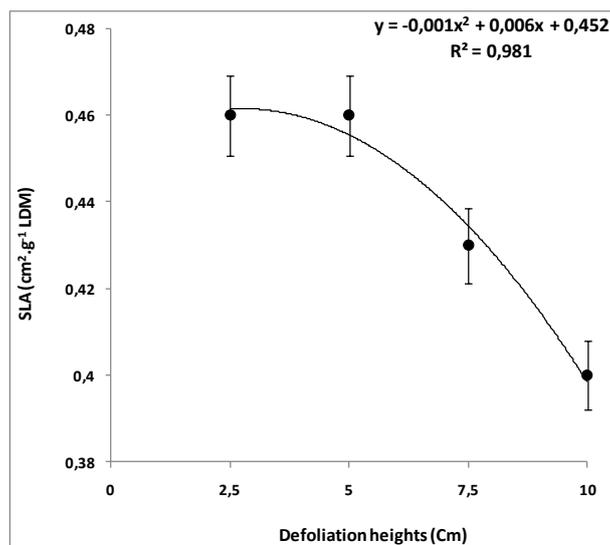
Leaves constitute the most active organ of the plant. They are the site of photosynthesis and transpiration. Plants growing under good conditions of water and mineral supply have as a priority the acquisition of an important leaf area, with the aim of an additional accumulation of biomass. To examine the impact of the defoliation height variation, we measured the leaf area in each harvest (figure 5). The results showed that the leaf area under the defoliation height of 5cm was significantly higher, than that in the extreme treatments (2.5 and 10cm).



**Figure 5.** Leaf area (LA) of berseem *Khadhraoui* under different defoliation heights (2.5 - 5 - 7.5 and 10cm). Each point represents the mean of 4 individual values. Intervals of confidence were calculated at  $\alpha = 95\%$ .

**Specific leaf area (SLA)**

The Specific leaf area, expressed in cm<sup>2</sup> per gram of dry matter of leaves is shown in figure 6. The statistical analysis of the SLA shows a significant difference between the defoliation heights of 2.5 and 5cm, on one hand and those realized at 7.5 and 10cm, on the other hand.



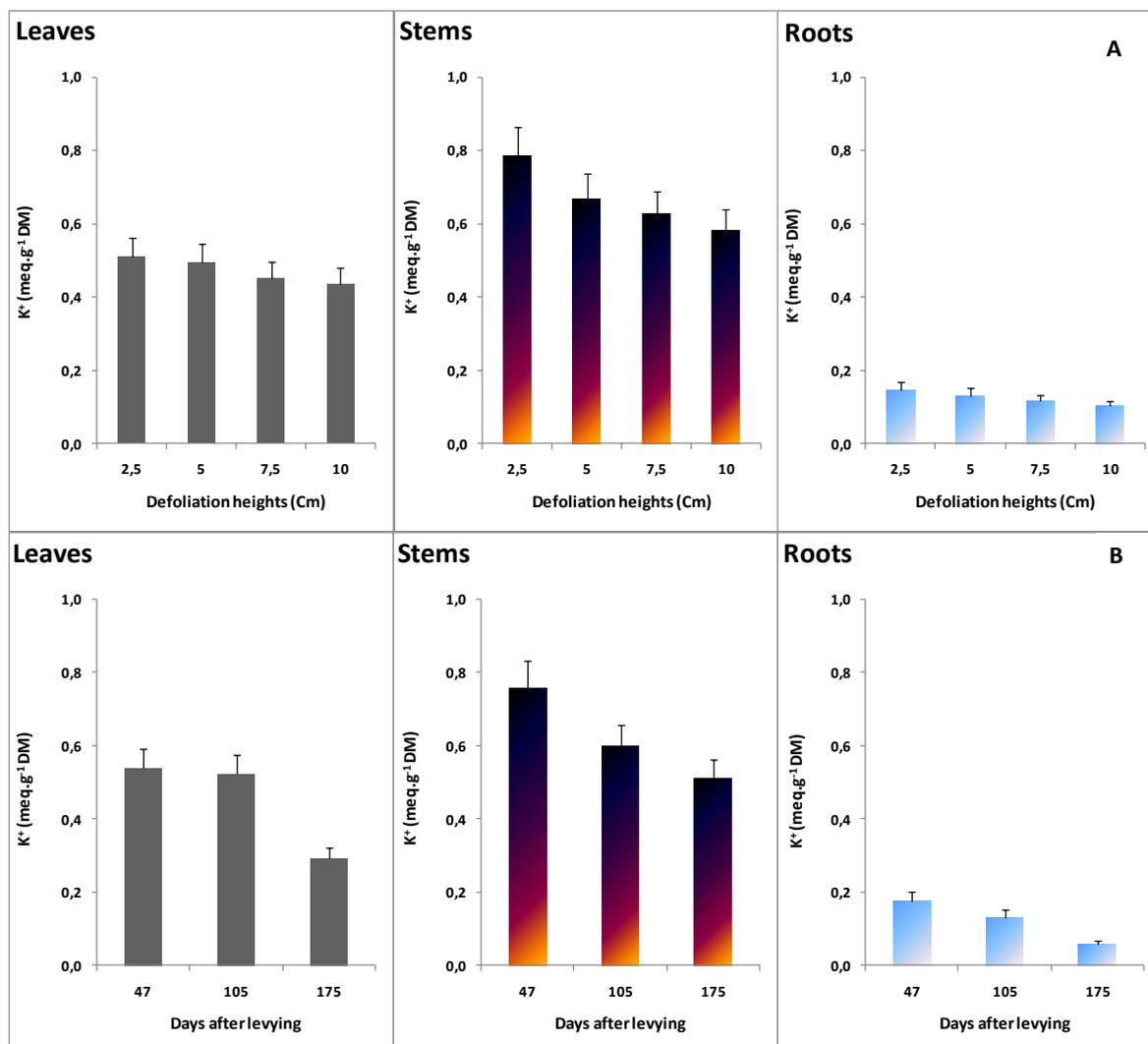
**Figure 6.** Specific Leaf area (SLA) of berseem *Khadhraoui* under different defoliation heights (2.5 - 5 - 7.5 and 10cm). Each point is the mean of 4 individual values. Intervals of confidence were calculated at  $\alpha = 95\%$ .

#### **Mineral contents in the organs of the berseem khadhraoui**

We studied the variation of the  $K^+$ ,  $Ca^{2+}$  and the inorganic phosphorus (P) distributions in the biomass of roots, stems and leaves of the berseem *Khadhraoui*, harvested at different defoliation heights. The defoliation which constitutes in itself a mechanic stress, affects the balance of source-sink, as a result of a decrease in the photosynthesing leaves. The defoliation leads to a modification in the activity of the ionic demands in the regenerated vegetative organs. The balances in leaf and stem transports and the root accumulation of the major cations ( $K^+$  and  $Ca^{2+}$ ), under different defoliation heights were studied at the beginning, in the middle and at the end of the experiment, respectively after 47 - 105 and 175 days of the levying.

#### **Potassium content**

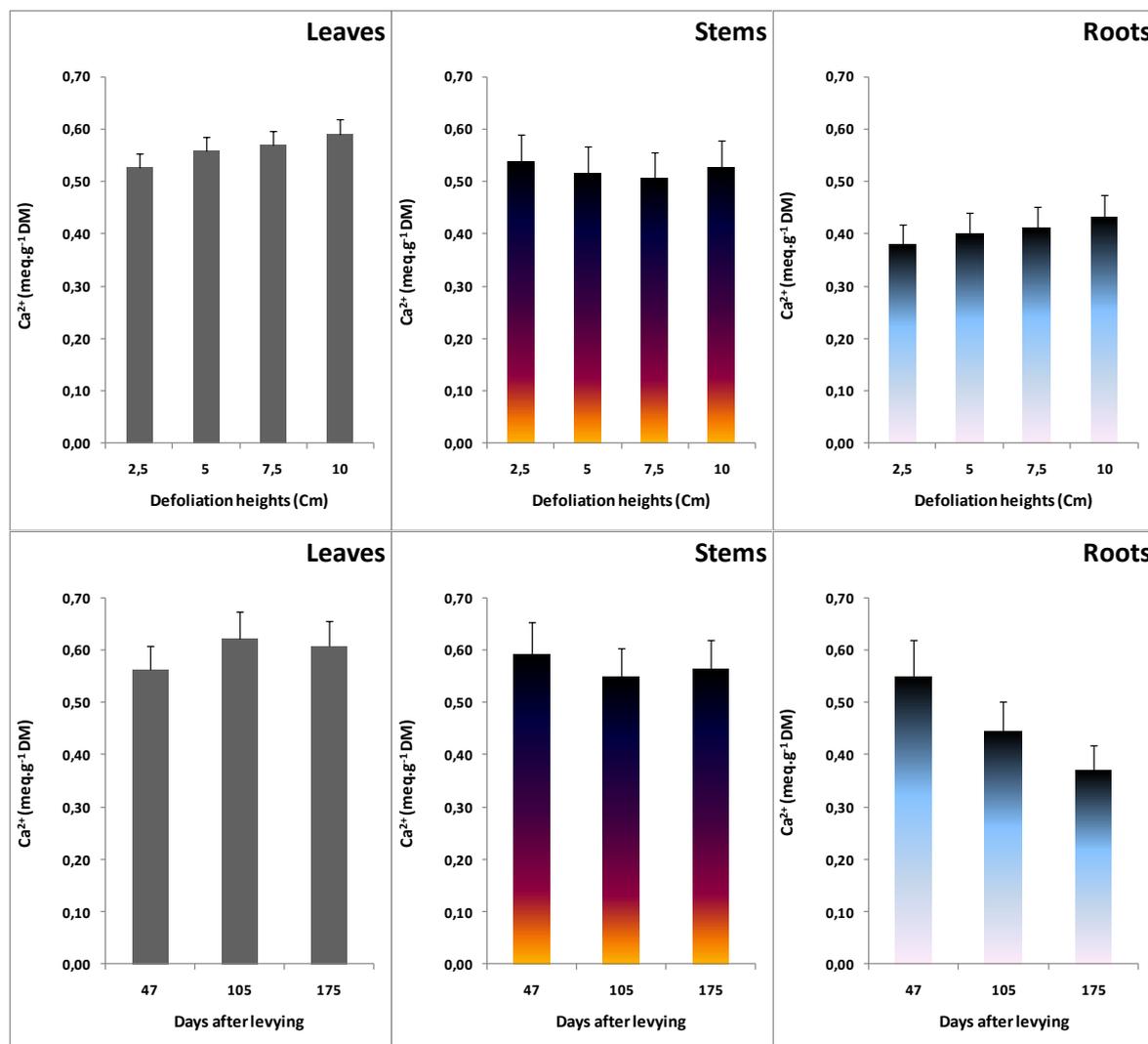
Figure 7A represents the variation of potassium contents in roots, stems and leaves according to the defoliation heights (2.5 - 5 - 7.5 and 10cm). Results showed that the  $K^+$  contents in the stems and leaves are significantly higher than those in the roots. For all organs (root, stem and leaf),  $K^+$  contents decreased significantly with the increase of the defoliation heights. That is  $K^+$  content under the defoliation height of 2.5cm was significantly higher than that of the stubble bases of 10cm height. We have also studied the variation of the  $K^+$  content at three different dates (the beginning, the middle and the end of the experiment). Figure 7B showed that  $K^+$  content decreased with the time. The decrease in  $K^+$  content in shoots (leaves and stems) and roots dry matter was the resulted from the depletion of the soil culture from the defoliation to another by the mass of dry matter harvested.



**Figure 7.** K<sup>+</sup> contents in leaves, stems and roots of berseem *Khadhraoui* under (A) different defoliation heights (2.5 - 5 - 7.5 and 10cm) and (B) (47 - 105 and 175) days after leving. Each point represents the mean of 4 individual values. Intervals of confidence were calculated at  $\alpha = 95\%$ .

### Calcium content

Figure 8A represents the variation of calcium contents in roots, stems and leaves according to the defoliation heights (2.5 - 5 - 7.5 and 10cm). Results showed no significant difference of calcium contents in the dry matter of roots, stems and leaves between the defoliation heights. This can be explained by the soil high content in soluble active chalk which did not affected the Ca<sup>2+</sup> content in the different plant parts (table 1). The variation in the calcium content according to the duration of the experiment (number of days after the leving, figure 8B) showed that only the Ca<sup>2+</sup> contents in roots decreased significantly with time.

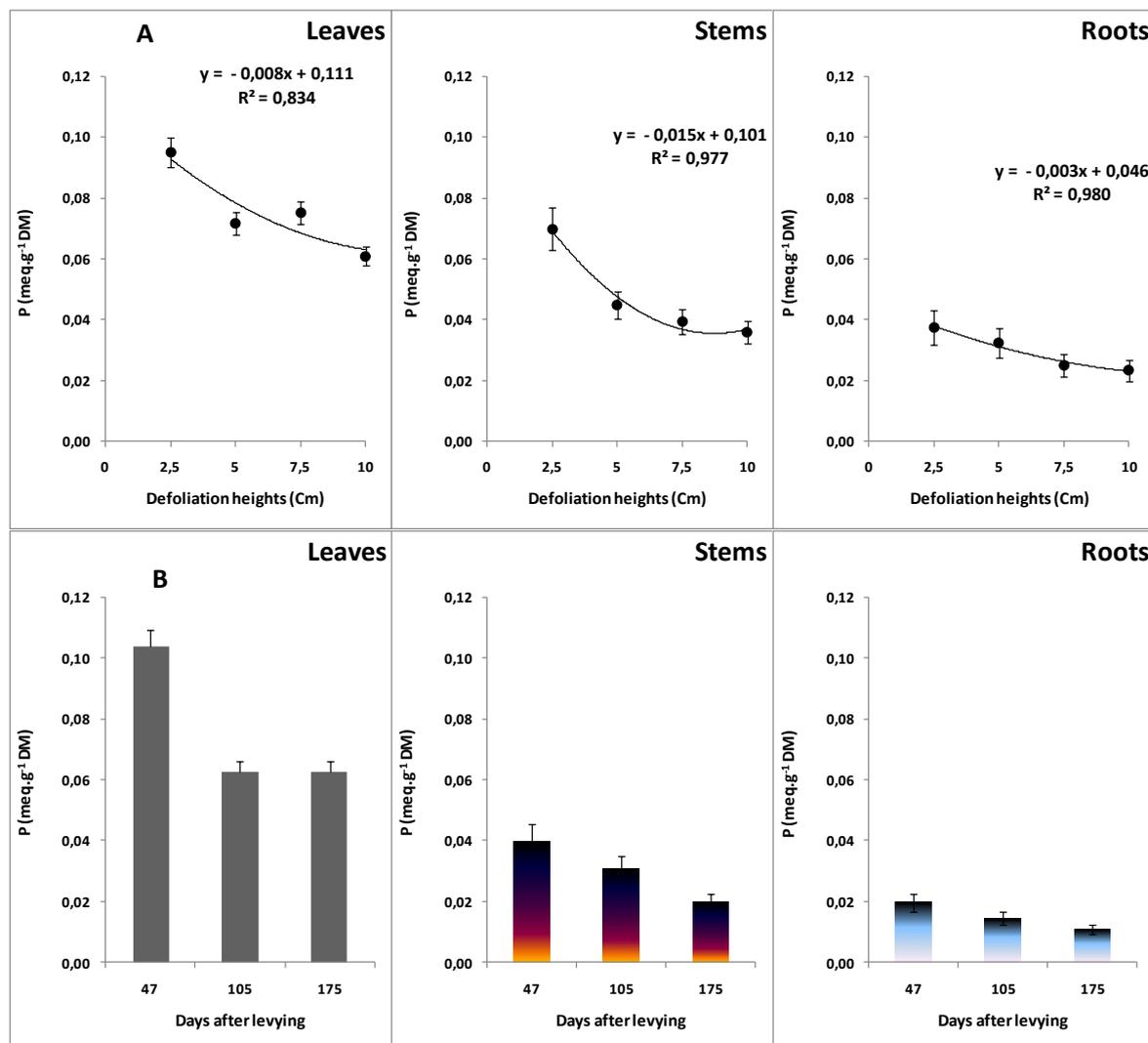


**Figure 8.** Ca<sup>2+</sup> contents in leaves, stems and roots of berseem *Khadhraoui* under (A) different defoliation heights (2.5 - 5 - 7.5 and 10cm) and (B) (47 - 105 and 175) days after leving. Each point is the mean of 4 individual values. Intervals of confidence were calculated at  $\alpha = 95\%$ .

### Phosphorus content

The variation in phosphorus content in leaves, stems and roots according to the defoliation heights is given in figure 9A. We observe that leaves, and to a less degree stems have higher phosphorus content compared to roots. The P contents decreased significantly with the defoliation heights, due to the dilution by the growth.

Similarly, the phosphorus content in leaves, stems and roots decreases with the sampling duration (figure 9B). Indeed, we observed a significant decrease in phosphorus contents in the dry matter of the three organs, between the beginning, the middle and the end of the experiment. This is the result of the depletion of the culture medium in phosphorus which is indispensable to the growth and the development of the plants.

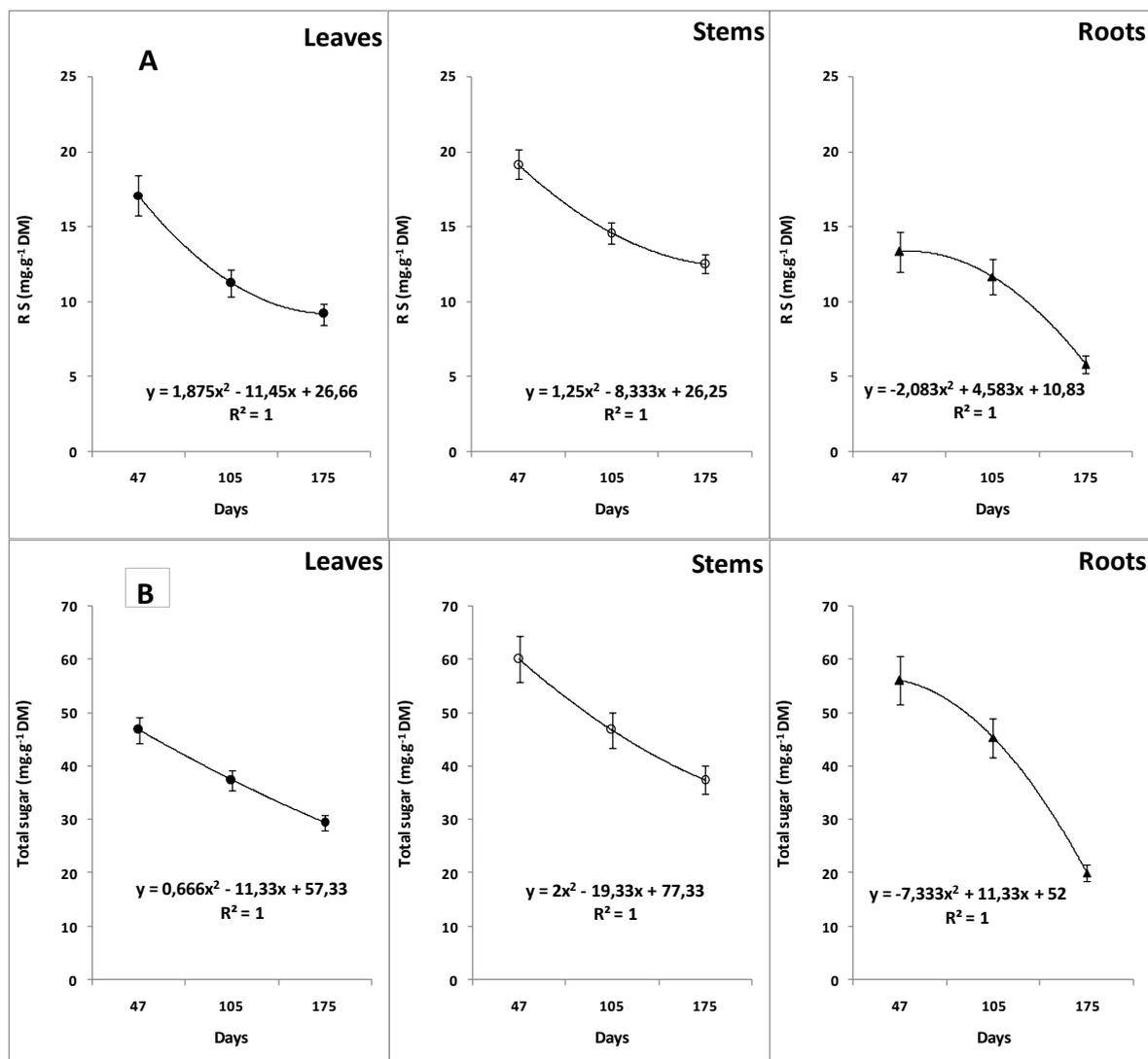


**Figure 9.** Phosphorus contents in leaves, stems and roots of berseem *Khadhraoui* under (A) different defoliation heights (2.5 - 5 - 7.5 and 10cm) and (B) (47 - 105 and 175) days after leving. Each point is the mean of 4 individual values. Intervals of confidence were calculated at  $\alpha = 95\%$ .

#### Carbohydrate contents in berseem organs

The reducing sugar contents in the three organs decreased significantly with the time. The decrease is very fast in roots, going from  $56\text{mg.g}^{-1}$  DM after 47 days of the leving to  $20\text{mg.g}^{-1}$  DM after 175 days of the leving which a decline of 56%, from the beginning of the experiment (fig. 10A).

As for soluble sugars, total sugars decreased according to the duration of the experiment in leaves, stems and roots of berseem *Khadhraoui*. The decrease of the total sugars in roots is very fast, going from  $13.33\text{mg.g}^{-1}$  DM after 47 days of the leving to  $5.83\text{mg.g}^{-1}$  DM after 175 days of the leving, that is a decline of 64% (fig. 10B).



**Figure 10.** (A) Reducing sugar (RS) and (B) total sugar contents in leaves, stems and roots of berseem *Khadhraoui*, at 47 - 105 and 175 days after levying. Each point is the mean of 4 individual values. Intervals of confidence were calculated at  $\alpha = 95\%$ .

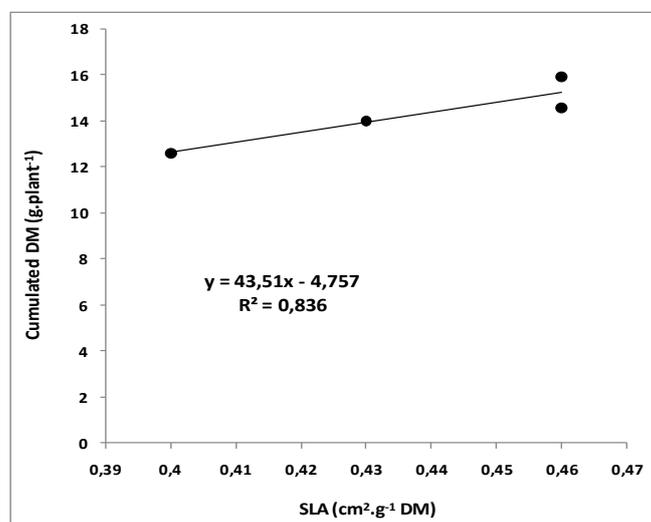
#### IV. Discussion

The objective of this work was to study the effect of the defoliation heights (2.5 - 5 - 7.5 and 10cm) on the growth, the mineral nutrition and the carbohydrate contents in the berseem *Khadhraoui*. The speed of the regrowth depends on the plant parts remaining below the the defoliation height level, which is depending on the quantity of green limbs left by the defoliation and on the quantity of soluble carbohydrates stored in roots at the defoliation time. Results showed that the cumulative dry material varies with the defoliation heights. So, in the sixth harvest, DM under the cutting height of 5cm is significantly higher, than in the other treatments.

Our results confirm those found by Burns et al., (2002) on the tall fescue (*Festuca arundinacea*) and by Leconte (2002) with English rye-grass (*Lolium perenne*); where they found that the dry matter yield was significantly higher under the shortest defoliation height. This can be explained by the fact that grasses can emit new tillers which replace those harvested, while the morphology of the berseem and alfalfa regrowth is constituted at the same time by parts left after defoliation and by the neoformed stems from the crown (Dufour et al., 1989). However, our results do not agree with those reported by Brathen and Odasz-albrigtsen (2000) in *Luzula arcuata*, Hendrickson and Berdahl, (2002) in *Thinopyrum intermedium* and by Onyeonagu and Ugwuanyi (2012) in *Panicum maximum*.

The leaf area (LA) per plant showed a depressive effect of the extreme defoliations: shorter (2.5cm) and higher (10cm), while the cutting heights at 5 and 7.5cm present a LA appreciably similar. Meuriot et al., (2004) reported that initial residual leaf area and initial N reserves influenced alfalfa regrowth and the residual leaf area had a greater effect on final forage production. Our results agree with those found by Giambalvo et al., (2011b) on berseem clover.

The specific leaf area (SLA) decreased with the increase of the defoliation heights and a significant difference is clear between plants defoliated at 2.5 and 5cm, on one hand and those harvested at 7.5 and 10cm, on the other hand. Figure 11 illustrated the correlation between the specific leaf area and the cumulated DM production. The correlation showed a highly significant linear relationship between DM and SLA ( $R^2 = 0.836$ ) which explains the improvement in the photosynthetic efficiency of the berseem clover *Khadhraoui*.



**Figure 11.** Correlation between cumulated dry matter and the SLA in the berseem *Khadhraoui* under different defoliation heights (2.5 - 5 - 7.5 and 10 cm).

The distribution of the shoot dry matter between leaves and stems allows the appreciation of the fodder quality of the produced biomass. The defoliation heights of 2.5 and 5cm have a fodder quality better than those of 7.5 and 10cm. Indeed, the short defoliations increase the formation of stems with many active leaves, while the highest defoliations stimulate the ramification of stems and activate the premature senescence of the old leaves left below the cutting level, resulting in an important mass of stems which depreciated fodder quality (photo 1).



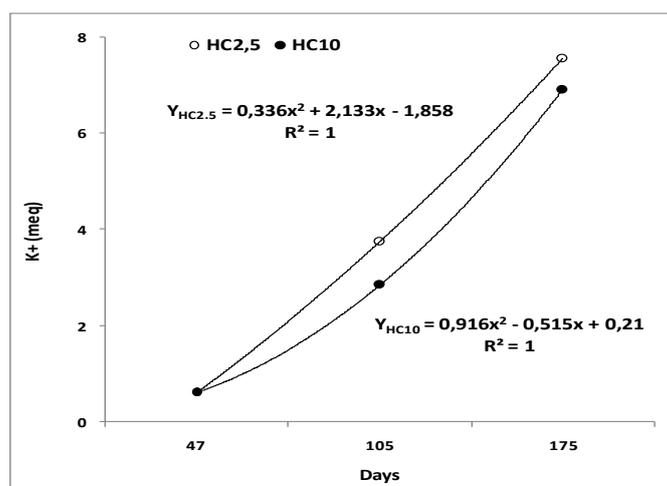
**Photo 1.** Effect of the cutting heights (HC) on the stubble bases of the berseem *Khadhraoui*

The distribution of photoassimilates between the shoots and roots (metabolites to be later reused in the regrowth) highlights the relation source/sink. Indeed, the shoots which contain leaves photosynthetically active produce photoassimilates or carbohydrates which a part migrate to the taproot and the crown to be stored and used later after defoliation to regenerate new leaves (Meuriot et al., 2004; Hopkins and Huner, 2008). In *Lolium perenne* and *Calamagrostis epigejos*, Louahlia et al., (1999), Gloser (2005); Kavanova´ and Gloser, (2005) and Gloser et al., (2007) found that amino acids and soluble proteins from roots and stubble bases were the most important sources of storage compounds for regrowth of the shoot.

The S/R ratio under the defoliation height of 5cm was the lowest, compared to other treatments. This showed that the root DM increases under the defoliation height of 5cm faster than that of the shoot DM, under the same treatment, which gives a shoot to root ratio significantly lower. Iannucci (2001) reported that just after cutting of two varieties of berseem, shoots are the main sinks, whereas roots and crowns represent the sources.

The effect of the defoliation height on the mineral nutrition of the berseem *Khadhraoui*, exposed to the mechanical stress of defoliation was studied in three different dates (beginning (47 d), middle (105 d) and the end (175 d) of the experiment). In these conditions, the tolerance will depend largely, on the root system to supply plants with an adequate mineral nutrition indispensable for their growth and for their development after defoliation.

Potassium is a critical nutrient for the growth, development, and persistence. Although the  $K^+$  content decreases according to the increase in the defoliation heights in the three organs of the berseem plants (fig. 7), the quantities of  $K^+$  in shoots (stems and leaves), exported by the harvested biomass at the most extreme treatments (2.5cm= short cutting) and the highest (10cm), increase linearly with the harvest date (fig. 12). This uptake by the plants of berseem depletes the culture medium in  $K^+$ , what is necessary to correct the soil deficiency in  $K^+$  with a supply of potassium amendment. In a study on the effect of defoliation interval and height on the growth of *Arachis pintoii*, Sinclair et al., (2007) found that the level of  $K^+$  in leaves decreased as the defoliation interval was extended and this effect was significant from 10 to 20 day intervals.



**Figure 12.** Quantity of potassium exported by berseem shoots under short (2.5cm) and high (10cm) defoliation at 47 - 105 and 175 days after leveying.

For calcium, results do not show any significant difference between the defoliation heights in stems and leaves (fig. 8). This is probably that the soil culture is very rich in active chalk (tab. 1). The phosphorus decreased significantly with the cutting heights and the time of the harvest (beginning, middle and the end of the experiment) in the three organs of the berseem *Khadhraoui* (fig. 9).

The study on the effect of defoliation heights on the growth and the productivity of biomass highlights the importance of the soluble sugar in the berseem *Khadhraoui* to regenerate shoots via the rémobilisation of the soluble carbohydrate reserves in crown and taproot. Indeed, the decrease of the soluble sugar contents in three organs of the berseem according to time shows a severe decline of such component in the root system. Teixeira et al., (2007) related the importance of the accumulation and depletion of N and C reserves in alfalfa crown and taproots in the shoot growth rate, mainly after defoliation and during early-spring regrowth.

#### Acknowledgements

This study was performed with the support of the Tunisia National Institute for Agricultural Research (INRAT) and the "Faculté des Sciences de Tunis".

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