RESEARCH ARTICLE Legume Research- An International Journal, Volume 44 Issue 6: 634-640 (June 2021)

# Humidity Restriction, High Night Temperature and their Combination, during Post Flowering on Common Bean (*Phaseolus vulgaris* L.) Canopy and Pod Senescence

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10.18805/LR-592

## ABSTRACT

**Background:** Common bean (*Phaseolus vulgaris* L.) grain filling is vulnerable to drought and heat stress. The objective of the study was to evaluate the effect of humidity restriction, high night temperatures and their combination in common bean.

**Methods:** The plants were grown in pots at maximum field capacity (100% FC) until the grain filling began. Afterwards, maintained until harvest at: 1) 100% FC, 2) 50% FC at ambient day/night temperature (AT), 22.58°C/16.94°C, 3) 100% FC with high night temperature (HNT) 21.47°C and 4) 50% FC with HNT (combined stress). Evaluations included phenotypic assessment using red-green-blue color segmentation in: green (healthy), yellow (senescence) and brown (necrotic).

**Result:** The combined stress in the cv. Rosa Bufa significantly and synchronously accelerated leaves and pods senescence. In contrast, in leaves of cv. OTI, the loss of green color began several days earlier than in pods. The effect of HNT and combined stress depends on common bean cultivar.

**Key words:** Common bean, Drought, High night temperature, Phenotyping, Senescence, Stay green.

## INTRODUCTION

The worldwide area cultivated with legumes is one tenth of that destined to the cultivation of cereals; furthermore, most of that area is cultivated under rainfed and few inputs compared to that of cereal cultivation. As a result, the yield of legumes is a quarter (0.86 t ha$^{-1}$) of that of cereals (3.6 t ha$^{-1}$). The common bean (*Phaseolus vulgaris* L.) is the main legume consumed in various regions of the world, it is the case of Latin America and Africa (FAO 2020). High temperatures (close to or higher than 35°C) and water stress during flowering and the establishment of the pods cause the abortion of inflorescences and even pods in early stages of development (Salcedo 2008). Projections indicates that by the end of the 21st century the mean global ambient temperature will increase between 1.5 and 4.5°C (IPPC 2013). The legume pods are fruits with two pod walls or valves that develop from a compressed ovary and protect the seeds during development (Clavijo *et al.* 2013), which will affect the common bean yield.

The legume pod walls perform photosynthesis, metabolize compounds, participate in the sink:source- relation and nutrients translocation to the seeds (Bennett *et al.* 2011). In chickpea (*Cicer arietinum* L.), drought and heat stress reduces water content, accelerates wilting, photosynthesis and assimilates production with respect to each separate stress (Awasthi *et al.* 2017). In lentil (*Lens culinaris* Medik), the combined stresses reduce seed starch accumulation, because the drastic inhibition of enzymes synthesize it, compared to individual stresses (Sehgal *et al.* 2018).

The dynamics of changes in source tissues, *i.e.* leaves and pod walls, can be evaluated with phenotypic, physiological and biochemical methods and non-destructive ones, such as those using RGB (Red, Green and Blue) (Fernandez-Gallego *et al.* 2018). The evaluation of stress on plants includes growth variables (morphometric analysis) and the permanence of the green color of photosynthetic tissues, known as 'Stay Green', which is defined as the permanence or decay to senescence of the green color (Thomas and Ougham, 2014) and is a character related to demand in seed production. Stay green evaluations have been applied in legumes like pea (*Pisum sativum*) (Quirós *et al*. 2019), soybean (*Glycine max*)

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**How to cite this article:** Cayetano-Marcial, M.I., Peña-Valdivia, C.B., García Esteva, A., Jiménez Galindo, J.C., Galván Escobedo, I.G. and Padilla Chacón, D. (2021). Humidity Restriction, High Night Temperature and their Combination, during Post Flowering on Common Bean (*Phaseolus vulgaris* L.) Canopy and Pod Senescence. Legume Research. 44(6): 634-640. DOI: 10.18805/LR-592.

**Submitted:** 08-10-2020 **Accepted:** 25-01-2021 **Online:** 24-04-2021

(Yuang *et al.* 2019) and chickpea (*Cicer arietinum* L.) (Joshi *et al*. 2016).

The objective of this study was to evaluate the impact of water restriction, high night temperatures (HNT) and their combination during post-flowering, on the senescence of the canopy on common bean cultivars with high potential for genetic improvement programs. Rosa Bufa is produced by smallholder farmers in regions of north of Chihuahua, México. The production is 1.5 t ha$^{-1}$ (Jiménez Galindo and Acosta Gallegos, 2013). The cultivar OTI was developed in Colegio de Postgraduados (COLPOS), Estado de Mexico and is used for consumers of the region. The production is 2.7 t ha$^{-1}$ (Estrada *et al.* 2004).

Phenotyping tools show great potential for successful breeding for resistance to drought and heat and selection for tolerance to abiotic stress. The hypothesis is that the response to the water deficit, HNT and its combination is intrinsically associated with the vegetative vigor or "Stay Green" of leaves and pods, is dependent on cultivar and is indicative of photosynthetic activity that alters the dynamics of senescence and translocation of biomass to seeds. This study´s results may be a valuable source of information which could be used for breeding in common bean of the other legume species.

## MATERIALS AND METHODS

### Biological material

The cultivars Rosa Bufa (Jiménez Galindo and Acosta Gallegos, 2012) and OTI (Estrada *et al.* 2004) were evaluated. Both cultivars exhibit type II growth habit (CIAT, 1982) Rosa Bufa seeds are produced by smallholder farmers in many region of mountains to the north of Chihuahua, México. The cultivar OTI was developed in Colegio de Postgraduados (COLPOS) and is produced in plots for consumers of the region. http://www.colpos.mx/semillas/frijoloti.html. The research period was from August to December 2018 in COLPOS in a greenhouse at the Colegio de Postgraduados Campus Montecillo. Montecillo. Texcoco. Estado de México. México. (19°27′ 40″ N, 98°54′ 19″ W and 2353 m altitude).

### Experimental design

The experimental design was of plots divided with random blocks and eight treatments (two cultivars, two water conditions and two temperature regimes). The experimental unit was a plant and five repetitions per treatment were evaluated. The plants were daily watered to field capacity (~100% FC) until the beginning of the pod filling (R8 growth state; CIAT 1982). Subsequently, the plants were separated into four groups: group 1, continuous irrigation until harvest (100% FC) at room temperature in greenhouse (ambient day/night temperature (AT), group 2, 50% reduced irrigation until harvest + AT, group 3, with 100% FC+HNT and group 4, 50% FC+HNT. The HNT values increased from 18-25°C ambient temperature, from 3h to 5h, respect the AT. The 50% FC condition was obtained through irrigation suspension; moisture monitoring *via* gravimetric showed that the substrate should contain 0.09 mL water g$^{-1}$ of soil.

The HNT condition was obtained in a unicel box (1.20 mx 4.5 m and 0.50 m; width, length and height), inside the greenhouse; electric heaters programmed with an automatic time recording system (timer) and those of daily temperatures inside and out of the box were recorded by a data logger (HOBO, USA).

### Imaging phenotyping analysis

The plants images were obtained using the Scanalyzer PL phenotyping platform (LemnaTec GmbH, Aachem, Germany). The lateral images (Fig 1) of each plant were obtained from three planes with 0°, 90° and 180° orientations. The treatments were photographed at beginning and at 0, 7, 18, 27 and 34 days after suspending irrigation and HNT. The images obtained and analyzed totaled 600 (Fig 1).

### Image analysis

Color segmentation was calculated from the images of the plants at senescence. Each image was processed using the LemnaGrid software (LemnaTec GmbH, Aachem, Germany) (Fig 2). At the same time, these colors relate to the physiology and phenology of the tissues (Padilla-Chacón *et al.* 2019).

### Yield and yield components

The plants were maintained until completing their reproductive stage and then dried biomass of the leaves, stem, pod walls and seeds was determined after an 80°C drying period for four days. The harvest index and pod harvest index were calculated according (Escalante Estrada and Kohashi Shibata, 2015).

### Statistical analysis

Statistical analysis included the ANOVA and multiple means comparison between treatments *via* the Tukey method ($\alpha = 0.05$).

## RESULTS AND DISCUSSION

### Phenotyping analysis

At the beginning of the pod filling the percentages of each green, yellow and brown color of the plants of the eight treatments were similar to each other; the green color of the plant canopy area was close to 100% (Fig 2A-B; 3A-B). Seven days after the beginning of the pod filling senescence in the cv. OTI was absent; this was independent of the water condition and night temperature (Fig 2A, 3C). Also plants of the cv. Rosa Bufa with 100% FC (groups 1 and 3) showed no severe senescence evidence, regardless of night temperature. In contrast, in the same 7-day period, the humidity restriction at 50% FC+HNT significantly accelerated senescence (Fig 2B, 3D). It was observed a decrease of more than 50% of the green color and a proportional increase in the yellow percentage. However, with 50% FC + HNT in this cultivar the green color remained higher than 50%, compared to the equivalent HNT treatment.

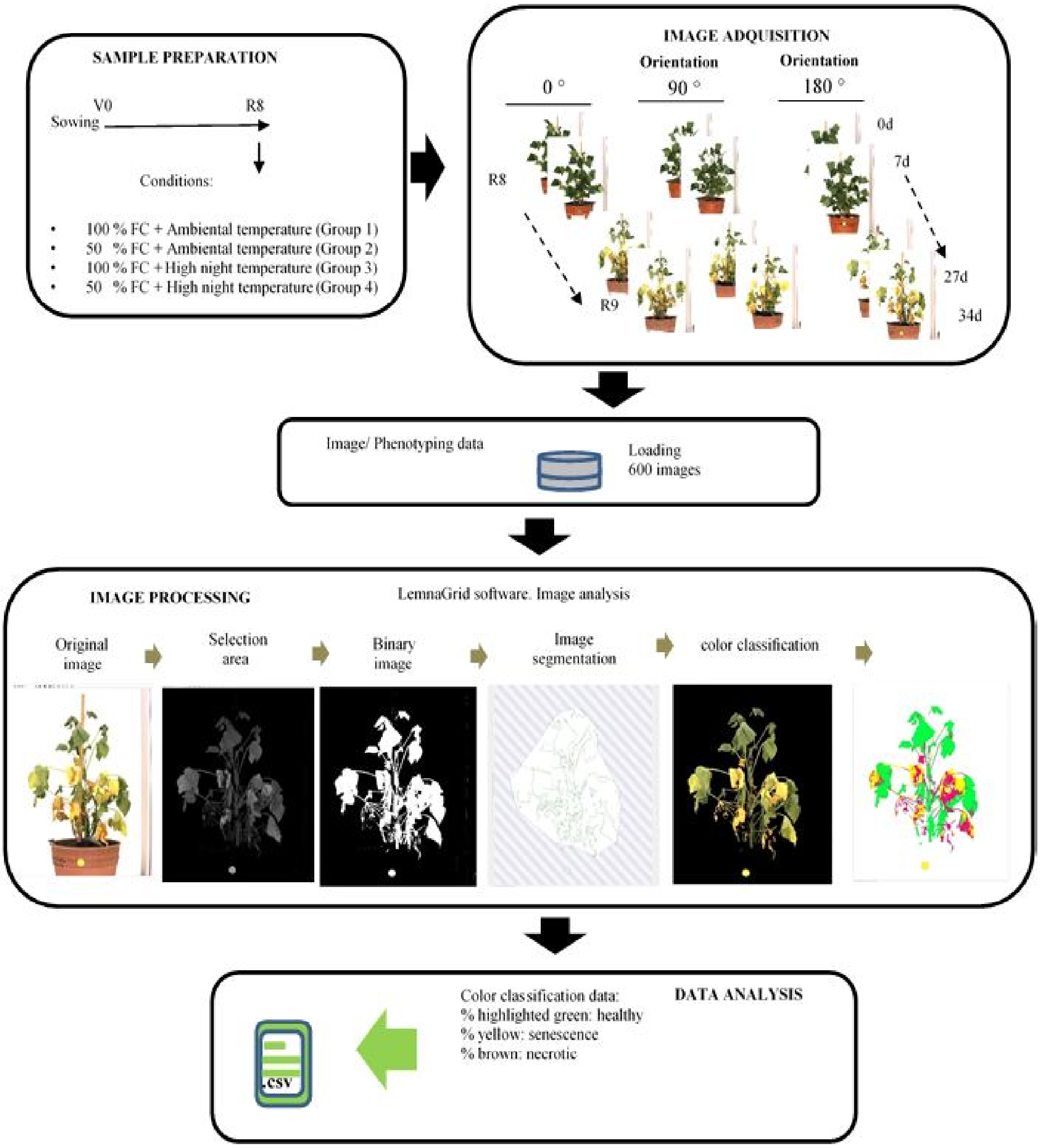


**Fig 1:** Example of Potential Applications of Phenotyping with Red–Green–Blue (RGB) Images Produced by Scanlyazer PL digital camera. The workflow describes the sample preparation in four groups of plants cv. OTI and Rosa Bufa in R8 stage: Group 1: irrigation maintaining 100% field capacity (FC) and ambient day/night temperature (AT), 22.58°C/16.94°C, Group 2: 50% FC + AT, Group 3: 100% FC and high night temperature (HNT) 21.47°C and Group 4 with 50% FC + HNT (combined stress). *n* = 5.

Eighteen days after the beginning of the pod filling the percentage of color in group 1 of the cv. OTI remained virtually unchanged (Fig 2A, 3E). In contrast group 1 of cv. Rosa Bufa in the same 18-day period decreased its green color by 60% and increased the yellow color by 30% and brown 30%, color associated with senescence and necrosis. The green color in group 3 of cv. OTI was significantly decreased (33 and 25%) respect to day zero and its group 1 (Fig 2B, 3F); the decrease was accompanied by a threefold increase in the colors associated to senescence and necrosis. In the cv. Rosa Bufa case, the effect of HNT during 18 days of pod filling was not evident as a plant color change.

The effect of HNT independent of water restriction (group 3), 27 days after the beginning of the pods filling, was significant in both cultivars. The combination of moisture restriction and HNT after 27 days significantly increased the

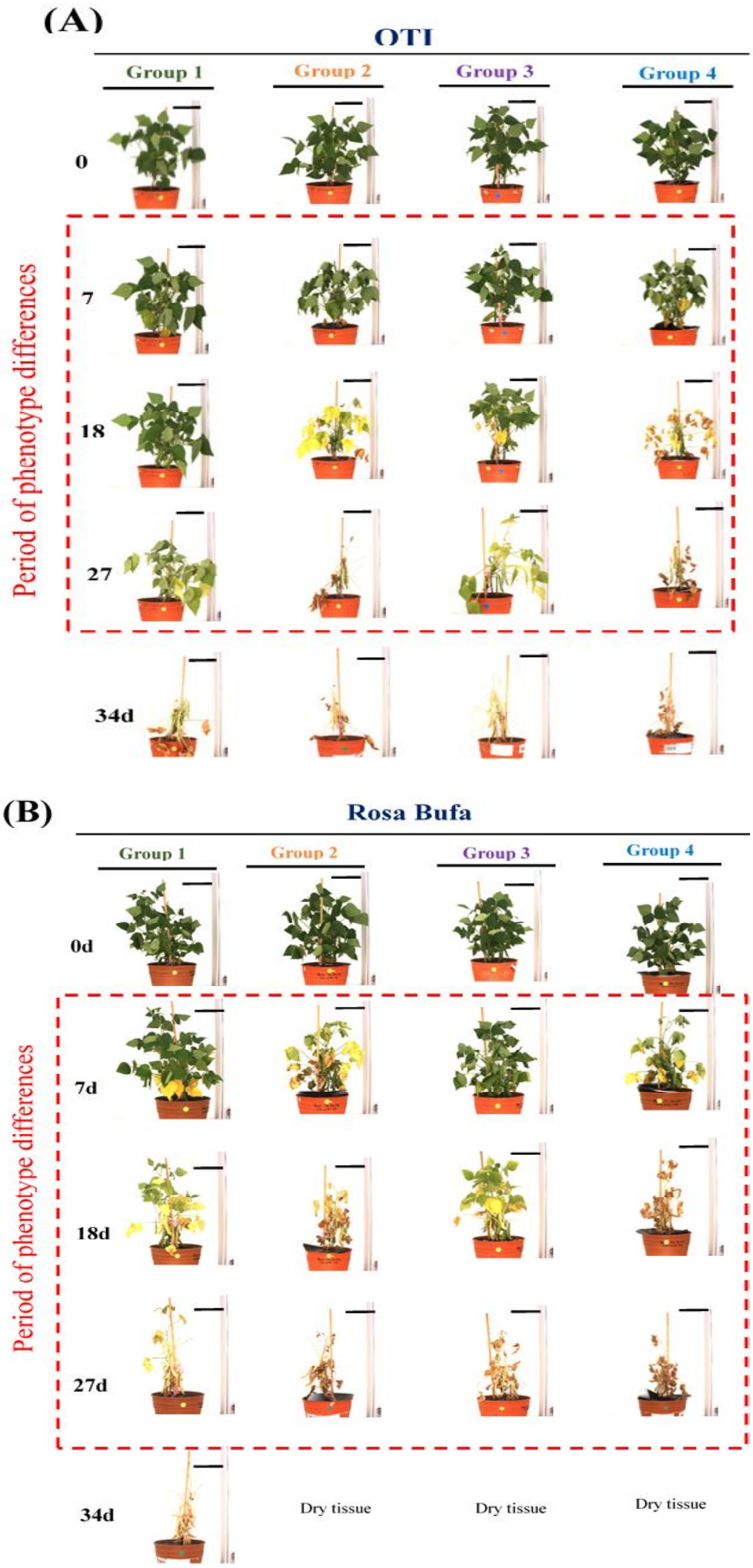


**Fig 2:** RGB images representing the effect of soil moisture and ambient temperature on common bean (*Phaseolus vulgaris* L.) plants of A. cv. OTI and B. Rosa Bufa during its reproductive stage. Groupl 1: irrigation maintaining 100% field capacity (FC) and ambient day/night temperature (AT), 22.58°C/16.94°C, Group 2: 50% FC + AT, Group 3: 100% FC and high night temperature (HNT) 21.47°C and Group 4 with 50% FC + HNT (combined stress). The bar corresponds to 10 cm.

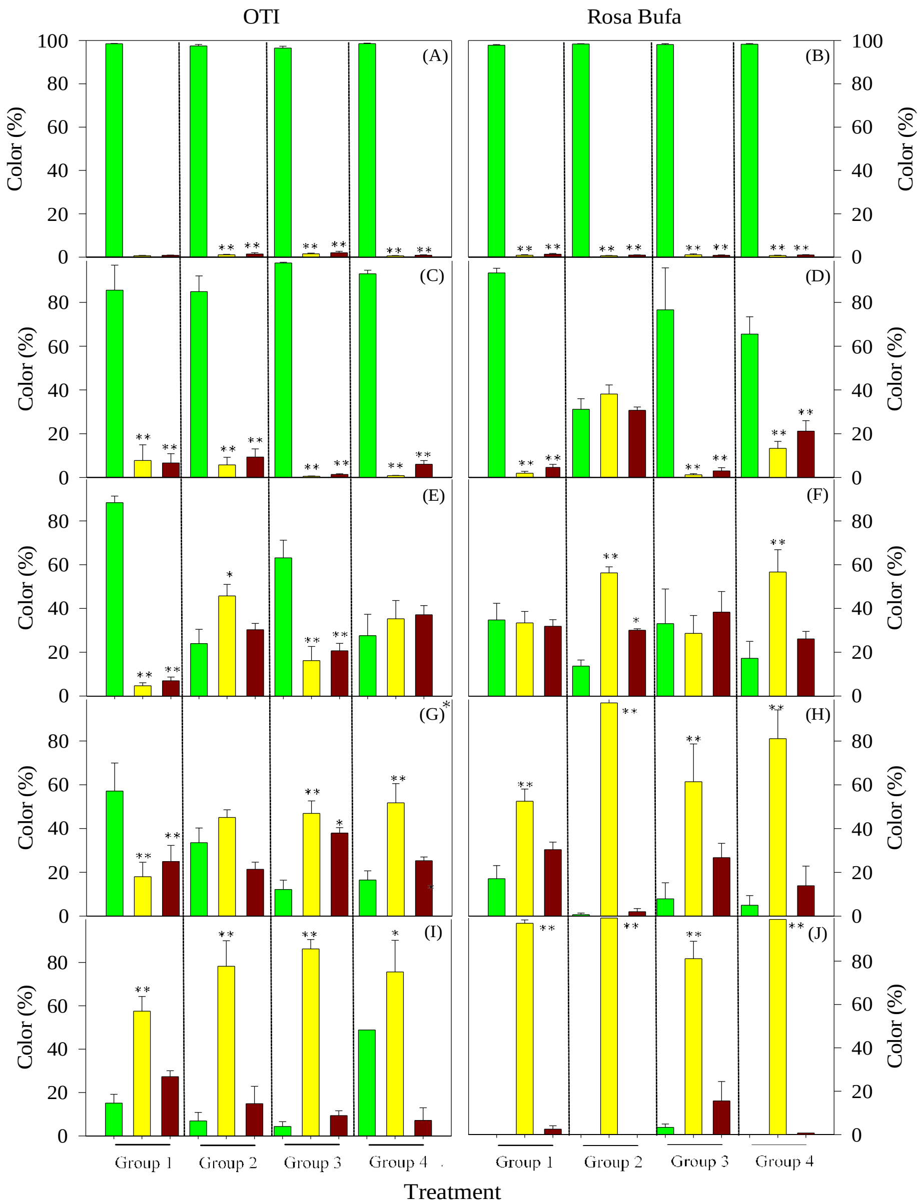


**Fig 3:** Phenotyping (+ se) of plants of common bean (*Phaseolus vulgaris* L.) from the OTI and Rosa Bufa cultivars, based on color (green: healthy, yellow: senescent and brown: necrotic) at the beginning (A-B) of the pods filling and 7 days (CD), 18 days (EF), 27 days (GH) and 34 days (IJ) after its beginning. ($n$ = 6). *P<0.05, **P<0.01.

senescence of both cultivars. In cv. OTI the effect was as a significant loss of 50% of the green color and a 27% increase in senescence respect to its group 1 (Fig 2A, 3G). In contrast, the decrease in the green color in cv. Rosa Bufa was severe with high percentage of necrotic tissue (Fig 2B, 3H). After 34 days, the cultivars contrasted in their natural senescence (group 1) and in the reaction to the moisture deficit (group 2) and its combination with HNT (group 4). The cv. OTI showed significantly higher green color, compared to its group 1 (Fig 2A, 3I), because its pods were maintained green. In this condition the cv. Rosa Bufa maintained total senescence compared to its respective group 1 (Fig 2B, 3J). Several non-destructive and high-performance phenotyping systems to analyze morphometric changes and color segmentation in plants have been developed (Khan, 2018; Ganiga *et al.* 2019). Our results exhibited the efficient detection of traits between common bean phenotypes during senescence process associated to chlorophyll degradation to colorness (Padilla Chacón *et al.* 2019). Leaf senescence is a relevant yield-related characteristic (Kim *et al.* 2018). Thus, the phenotyping of the effect of heat-drought stress will make it possible to advance in the molecular mechanisms identification involved in legumes senescence. The *Tnt1* mutant of *Medicago truncatula* maintains green leaves during stress (Zhou *et al*. 2011). The cv. OTI in the present study showed that pericarp consume reserves depending

**Table 1:** Mean values of traits measured at maximum field capacity (100 % FC) and water restriction (50% FC) of cultivars OTI and Rosa Bufa at ambient day/night temperature (AT) and high night temperature (HNT).

| Cultivar | Humidity level (% FC) | Environment | Seed (g plant$^{-1}$) | Pod (g plant$^{-1}$) | Stem weight (g plant$^{-1}$) | Canopy weight (g plant$^{-1}$) | Harvest index (%) | Pod harvest index (%) |
|---|---|---|---|---|---|---|---|---|
| OTI | 100 | AT | 16.6 ± 0.5a | 5.7 ± 0.3a | 2.8 ± 0.1a | 7.9 ± 0.4a | 50.0 ± 1.14a | 74.2 ± 0.9a |
| | 50 | | 8.8 ± 0.4b | 2.3 ± 0.1b | 2.3 ± 0.1a | 7.9 ± 0.6a | 41.0 ± 2.0b | 78.7 ± 0.5a |
| | 100 | HNT | 14.7 ± 1.0a | 5.1 ± 0.1a | 3.0 ± 0.2a | 7.7 ± 0.4a | 47.7 ± 2.2a | 73.6 ± 1.4a |
| | 50 | | 7.2 ± 0.2b | 1.9 ± 0.1b | 2.9 ± 0.3a | 8.0 ± 0.8a | 38.0 ± 1.2b | 79.0 ± 1.0b |
| Rosa Bufa | 100 | AT | 18.5 ± 0.9a | 6.2 ± 0.5a | 2.3 ± 0.0a | 6.3 ± 0.3a | 54.5 ± 0.9a | 74.5 ± 0.6a |
| | 50 | | 9.3 ± 0.5b | 2.8 ± 0.1c | 2.1 ± 0.0a | 6.3 ± 0.2a | 44.0 ± 2.0b | 76.2 ± 0.6a |
| | 100 | HNT | 15.3 ± 0.7a | 4.7 ± 0.0b | 2.2 ± 0.5a | 5.7 ± 0.6a | 54.7 ± 1.1a | 76.3 ± 0.5a |
| | 50 | | 8.0 ± 0.6b | 3.2 ± 0.2c | 2.2 ± 0.1a | 6.5 ± 0.4a | 40.0 ± 1.3b | 71.0 ±1.9b |

Means in the same column followed for lower case letters (a, b, c). The same letters are not significantly different at P<0.05.

on the culture conditions and the presence or absence of stress. At the same time, it revealed the importance of foliar senescence as a marker of plant's response to environment and genetic improvement.

### Harvest index

The harvest index modified in group 1 of cv. OTI and cv. Rosa Bufa was 50 and 54% (Table 1). Under HNT (group 3) the harvest indexes in both cultivars were similar to the standard ambient temperature. The humidity restriction led to the fall of 38-40% of harvest index in the cv. OTI and 42-44% in the cv. Rosa Bufa regardless of standard temperature (Table 1).

The pod harvest index under optimal conditions (groups 1) was 74-76% in both cultivars. In addition, it was similar to the standard condition with HNT (Table 1). The humidity restriction, under standard temperature and HNT, increased 5-6% compared to the treatment with 100% FC in the cv. OTI. These results indicate that water restriction increased the mobilization of photoassimilates from the pericarp to the seeds. In this regard, pods are indicative of yield (Assefa *et al*. 2013) and a criterion for successful selection of drought-tolerant genotypes.

Furthermore, as in other studies (Assefa *et al*. 2013; Montero-Tavera *et al*. 2008), the results confirmed that the pericarp protects the seeds and is a carbon source for them and that humidity restriction affects severely seeds filling; and this process response to stress was different among the evaluated cultivars. *E.g*., the pod harvest index of cv. Rosa Bufa, showed no significant difference respect to the effect of the irrigation restriction (groups 1 and 2); but, water restriction+HNT decreased 7% this index compared to the standard conditions (group 1) (Table 1).

## CONCLUSION

In combined stress, the changes in the leaves and pods resulted in loss of greenery synchronously and accelerated in cv. Rosa Bufa. In contrast, in leaves of cv. OTI, the loss of green color began several days earlier than in the pods. This effects indicate that there is diversity in the response to drought stress in beans, HNT and its combination; also, this response depends on the cultivation.

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