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RESPONSE OF SUNFLOWER (*Helianthus annuus* L.) TO NITROGEN AND POTASSIUM DEFICIENCY

M. El Midaoui[1], M. Benbella[1], A. Talouizete[2], A. Bervillé[3]

[1] *Ecole Nationale d'Agriculture DAAP, BP S/40, 50000 Meknès, Morocco*
[2] *Faculté des Sciences Sidi Mohamed Ben Abdellah, Dhar Mahraz, Fès, Morocco*
[3] *INRA-ENSA UR GAP, 2, Place P. Viala, 34060 Montpellier Cedex, France*

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SUMMARY

The experiment was carried out under greenhouse conditions. Three sunflower genotypes were tested: Oro 9 (a variety population) and two hybrids (Isostar and Flamme).

N and K deficiency affected significantly shoots and roots morphological parameters for all examined sunflower cultivars. Shoots appeared to be more sensitive than roots. Reduction were more pronounced for dry weight under N deficiency than under K deficiency.

Root volume play an essential role in water and mineral absorption. Under normal supply of N and K root volume vas highly correlated, to shoot dry weight (r=0.89), to root dry weight (r=0.84) and to total dry weight (r=0.86). Under No and Ko, reduction of root volume and root dry weight reflected growth inhibition caused by N and K deficiency. Root volume, under No and Ko, was highly correlated, to root dry weight (r=0.99 and r=0.97 respectively for No and Ko), to shoot dry weight (r=0.89 and r=0.96 respectively for No and Ko) and total biomass (r=-0.93 and r=0.99 respectively for No and Ko).

Total soluble sugars accumulation under No and Ko was observed at two pairs of leaves for all genotypes. The accumulation was highly and negatively correlated to root biomass (r=-0.98) under No. However, a weak and negative correlation (r=-0.16) between these two parameters was found for Ko. Total soluble sugars were also strongly and negatively correlated, to root volume for No (r=-0.98) and Ko (r=-0.99). Cultivars with high root total soluble sugars content seems to be more sensitive to N and K deficiency like Isostar.

Response of cultivars to N and K deficiency resulted in an accumulation of amino acids.

Genotype Isostar, whose morphological characters (except leaf area) were severely affected, was the most sensitive. On the contrary Flamme was the least sensitive because of its capability to maintain an adequate growth rate causing a small reduction of morphological parameters. Oro9 had an intermediate behaviour.

Key words: **Sunflower, mineral deficiency, morphological parameters, asimilate accumulation**

INTRODUCTION

Mineral deficiency effects on plants result in a reduction of their growth the intensity of which depends on the species and the deficient element. Radin and Boyer (1982) have reported that nitrogen deficiency reduced the rate of leaf emission. On the other hand, Hocking and Steer (1982) pointed out that, in addition to its drastic effect on shoot growth, mineral deficiency reduced floral initiation, total number of flowers, total number of achenes and consequently grain yield. Kuchenbuch *et al.* (1988) found that nitrogen deficiency reduced shoot growth of sunflower more than its root growth. Eghball (1993) reported that root morphology of corn was altered by nitrogen deficiency and that root branching was less developed.

Adequate supply of K to corn stimulated root proliferation and extension (Edward, 1981). Blanchet *et al.* (1973) studied root growth in relation to mineral nutrient and found that nutrient uptake was significantly correlated to root development.

The role of K in osmoregulation is obvious. It is involved in stomata functioning (Heller, 1977) and in water use efficiency (Blanchet *et al.*, 1982). This element tends to increase guard cell osmotic pressure by acting on stomata aperture. Ability of osmotic adjustment of a plant comes according to Blum (1988) from its ability to accumulate osmotic solutes at the symplastic level.

Mechanisms involved in the response of crop plants to mineral deficiency are numerous and complex. They take in account morphological, physiological and biochemical characters. These mechanisms interact at different levels of plant organization. The purpose of this research was, in the first step, to study a global behaviour of sunflower plants and to identify different mechanisms involved in the response of this crop to nitrogen and potassium deficiency.

MATERIALS AND METHODS

The experiment was carried out under greenhouse conditions. Three sunflower genotypes were tested: Oro 9 (a variety population) and two hybrids (Isostar and Flamme). Principal characters of genotypes are described in Table 1.

Table 1: Principal characters of tested genotypes

Genotypes	Observations	Wild species background
Oro 9	Moroccan population	bred in dry conditions
Flamme	Commercial F_1 hybrid	drought tolerant
Isostar	Commercial F_1 hybrid	drought tolerant

Genotypes were factorial combined with three nutrient solutions: Hoagland solution (control), nitrogen-free Hoagland solution and potassium-free Hoagland solution. A complete randomized design with three replicates was used. Germinated sunflower seeds were gently transplanted in a 200 ml polyethylene pot filled with quartz sand. Two to three holes were made at the bottom of each pot to avoid asphyxia. Pots were irrigated daily until harvest (two pairs of leaves). Amount of solution added daily to pots was calculated by gravimetric method. Pots were covered by aluminium foil to prevent direct evaporation.

At harvest (two pairs of leaves), shoot were separated from roots and the following measurements were made:

Morphological characters

Root were gently removed from the pot and thoroughly washed by tap water. They were gently blotted between two sheets of paper before being weighed to determine root fresh weight. Root length was determined by measuring length of the longest root of the plant. Root volume (RV) was determined by following the method of Musick *et al.* (1965). Roots were then oven dried at 80°C for 48^h and their dry weight was measured.

Total leaf area (TLA) was measured by an electronic planimeter Li3000 (Li COR, Nebraska, USA). Plant height was measured as the distance from the plant collar to the end of the youngest leaf. Collar diameter was measured with scaled adjustable spanner. Harvested samples were then oven dried at 80°C for 48^h and their dry weight was measured.

Biochemical analysis

Amino acids

Methods described by Hayman and Kar (1975) were adapted for amino acids determination. An 0.2 g of leaf fresh weight was put in a test tube containing 10 ml of 80% ethanol. Tubes were immersed in a water bath maintained at 80°C. When alcohol began to boil, tubes were removed frequently and dipped again in the bath to avoid alcohol evaporation. After ten minutes immersion in water bath, tubes were removed and let to cool at room temperature. An aliquot of 0.5 ml was put in a test tube, 5 ml of 50% ethanol were added and the test was shaken to mix the sample. Optical density of the solution was read at 570 nm. Leucine was used as standard for calculating total amino acids concentration.

Total soluble sugars

The method of Lewichki as modified by Durnete (1960) and simplified by El Midaoui and Benbella (1996) was used. An 0.1 g of fresh leaves were put in a test tube containing 3 ml of 80% alcohol. The sample was heated at 80°C for 30 min. Tubes were then cooled at room temperature. An aliquot of 2 ml was added to 4 ml of a reagent made of anthrone and sulfuric acid maintained at 0°C. Tubes were vigorously mixed before putting them in a water bath maintained at 92°C for 8 min. Tubes were removed and let to cool for 30 min. in ice. Optical density was red at 585 nm using a spectrophotometer (Perkin Elmer 55-B). Total soluble sugars were determined using glucose (ref. Labosi G 305) as a standard.

RESULTS

Effect of N and K deficiency on morphological parameters

Under non-limiting water conditions, N and K deficiency resulted, for all genotypes, in a significant reduction of different morphological parameters for both shoot and root. Reduction was more pronounced for shoots than for roots. The results showed a significant effect of variety and N and K deficiency on total leaf area, plant height, stem diameter and volume and root length.

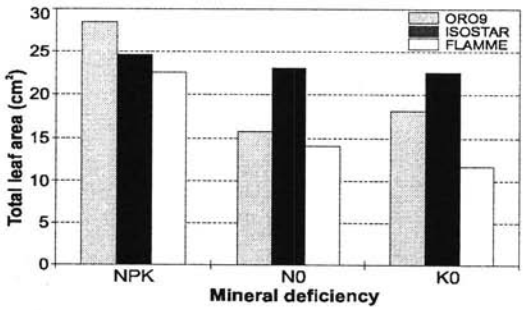


Figure 1: Effect of N and K deficiency compared to total grean leaf area of three sunflower genotypes

Total leaf area

Nitrogen and K deficiency had similar effects. They reduced TLA by an average of 30% compared with the control. Isostar had the highest TLA (23.1 cm^2 for No and 22.56 cm^2 for Ko).

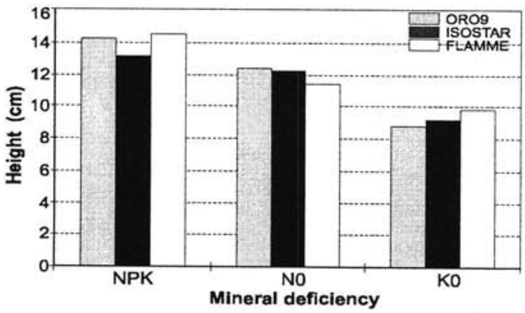


Figure 2: Effect of N and K deficiency compared to complete solution on height of three sunflower genotypes

Plant height

Results showed a reduction of 13.98% an 33.55%, respectively, for No and Ko (Figure 2). Oro 9 was the most sensitive genotype with a 30% reduction for No and 13% for Ko compared with the control.

Stem diameter

Under a given nutrient solution, no significant differences were observed among genotypes. Nitrogen and K deficiency induced a 33% reduction (Figure 3). With the complete nutrient solution Isostar had the highest stem diameter (39.4 cm) and Flamme the lowest (36.1 cm).

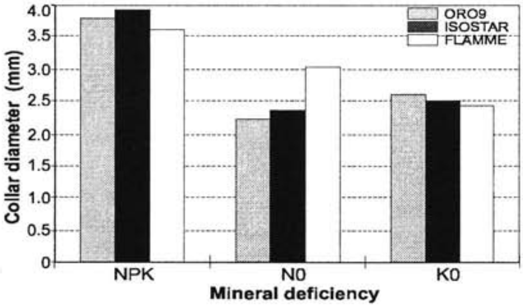


Figure 3: Effect of N and K deficiency compared to complete solution on the collar diameter of three sunflower genotypes

Root volume and root length

Nitrogen and K deficiency resulted in a net reduction of root volume and root length (Figures 4 and 5). Root volume was more affected by N and K deficiency than root length. Percent reduction of root volume was 62.35% for N and 37% for K against 26.83% and 19% for root length, respectively. Results showed also significant differences among genotypes. Under normal supply of N an K Flamme had the highest root volume (15.33 10^{-3} cm^3) and Isostar had the highest root length (19.2 cm).

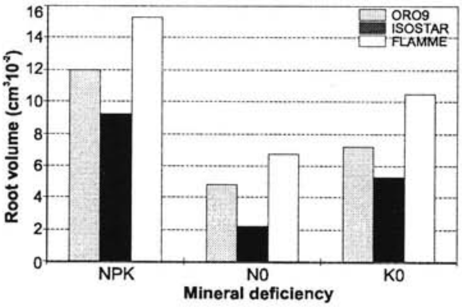


Figure 4: Effect of N and K deficiency compared to complete solution on root volume of three sunflower genotypes

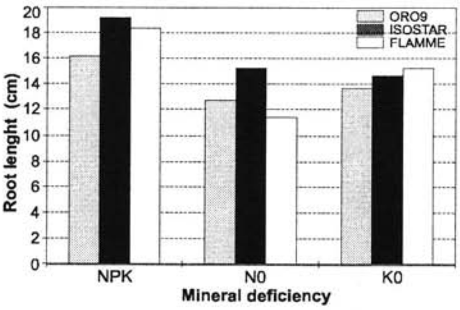


Figure 5: Effect of N and K deficiency compared to complete solution on root lenght of three sunflower genotypes

Dry weight

Shoot and root dry weight were drastically affected by N and K deficiency (Figures 6 and 7). Roots were more affected than shoots. Nitrogen deficiency reduced dry weight by 65% and 23% for root and shoot, respectively. Percent reduction caused by K deficiency was 34.35% for root and 10% only for shoot. Flamme had the highest root and shoot dry weight for all nutrient solutions.

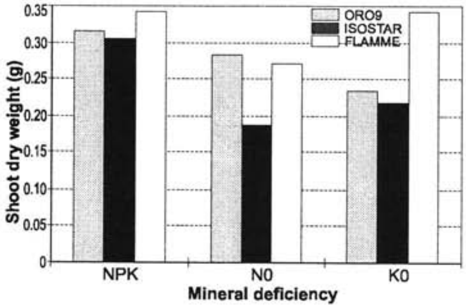


Figure 6: Effect of N and K deficiency compared to complete solution on shoot dry weight of three sunflower genotypes

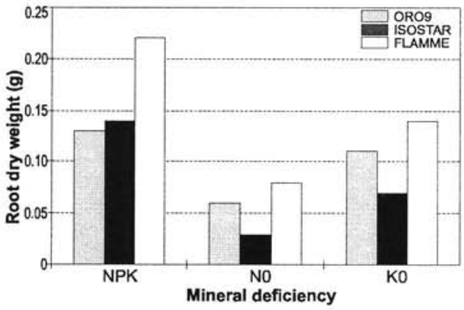


Figure 7: Effect of N and K deficiency compared to complete solution on root dry weight of three sunflower genotypes

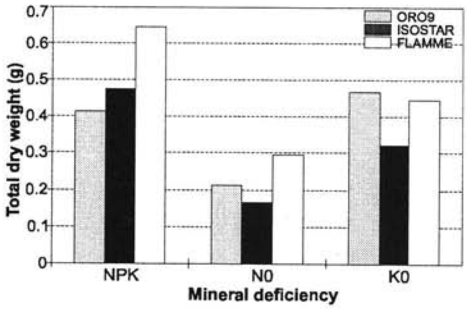


Figure 8: Effect of N and K deficiency compared to complete solution on total dry weight of three sunflower genotypes

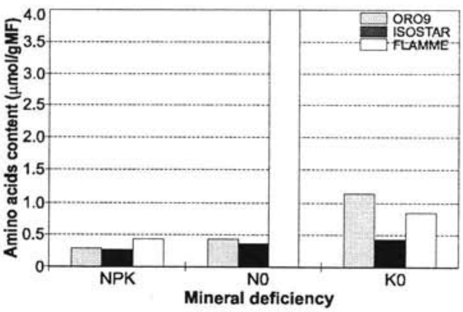


Figure 9: Effect of N and K deficiency compared to complete solution on amino acids content of leaves of three sunflower genotypes

Effect of N and K deficiency on amino acids and soluble sugars content

Nitrogen and K deficiency increased leaf amino acids (AA) content. Accumulation was more pronounced for roots (Figure 10). Percent increase of foliar AA was 122% and 243% respectively for No and Ko. Among genotypes Oro 9 had the highest leaf AA content. Under normal supply of N and K Isostar had the lowest leaf AA content and Flamme the highest. Isostar accumulated more AA in roots when irrigated with a complete or nitrogen-free solution. Oro 9 showed the same trend but with both No and Ko.

Total soluble sugars (TSS) content of leaves and roots of the genotypes increased under N and K deficiency (Figures 11 and 12). Total soluble sugars accumulated was higher for leaves than for roots. Percent increase of foliar TSS was 183% and 137%, compared with control, for No and Ko, respectively. Under nitrogen deficiency, Oro 9 had the highest leaf TSS and Isostar the highest roots TSS. For Ko Isostar accumulated more TSS in leaves while Oro 9 accumulated more TSS in roots.

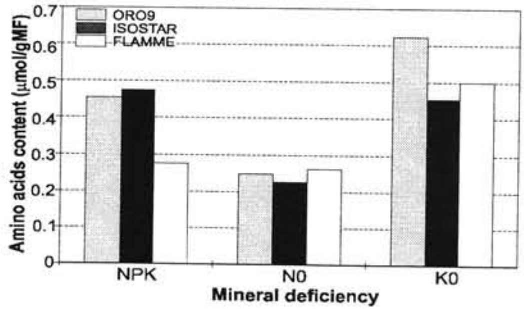
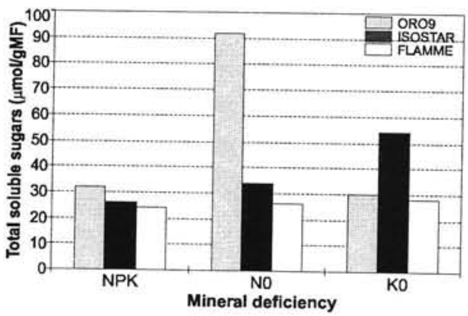


Figure 10: Effect of N and K deficiency compared to complete solution on amino acids content of root of three sunflower genotypes



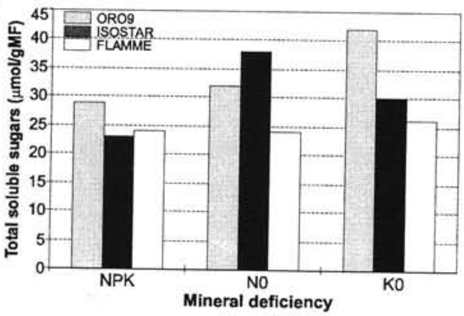


Figure 11: Effect of N and K deficiency compared to complete solution on total soluble sugars of leaves of three sunflower genotypes

Figure 12: Effect of N and K deficiency compered to complete solution on total soluble sugars of roots of three sunflower genotypes

DISCUSSION AND CONCLUSION

Results showed that N and K deficiency affected significantly shoots and roots morphological parameters for all cultivars. Shoots appeared to be more sensitive than roots. Reductions were more pronounced for dry weight under N deficiency than under K deficiency. De Raissac (1992) found the same results and reported that biomass reduction was often accompanied by an equivalent decrease in grain yield.

Concerning root morphological characters, root volume plays an essential role in water and mineral absorption. Results showed that under normal supply of N and K root volume vas highly correlated to shoot dry weight (r=0.89), root dry weight (r=0.84) and total dry weight (r=0.86). Under No and Ko, reductions of root volume and root dry weight reflected growth inhibition caused by N and K deficiency. Similar results were reported for other stresses such as water stress on wheat (Benlaribi *et al.*, 1990), barley (Khaldoun *et al.*, 1990), cotton (Huck *et al.*, 1970), sugar beet (Wendell *et al.*, 1973) and sunflower (El Midaoui *et al.*, 1998b). Root volume reduction limits root permeability and consequently absorption (Cruizat, 1974) and hence biomass production. Therefore, in this experiment, root volume, under No and Ko, was highly correlated to root dry weight (r=0.99 and r=0.97, respectively, for No and Ko), to shoot dry weight (r=0.89 and r=0.96, respectively, for No and Ko) and total biomass (r=-0.93 and r=0.99, respectively, for No and Ko).

Total soluble sugars accumulation under No and Ko was observed at two pairs of leaves for all genotypes. The accumulation was highly and negatively correlated to root biomass (r=-0.98) under No. However, a weak and negative correlation (r=-0.16) between these two parameters was found for Ko. Total soluble sugars were also strongly and negatively correlated to root volume for No (r=-0.98) and Ko (r=-0.99). Cultivars with high root total soluble sugars content seem to be more sensitive to N and K deficiency, like Isostar.

There was no clear relationship between leaf content and shoot biomass. However, leaf total soluble sugars were strongly and negatively correlated to root biomass (r=-0.93) and total biomass (r=-0.93).

Response of cultivars to N and K deficiency resulted in an accumulation of amino acids. Hackett *et al.* (1965) found the same results for barley under K deficiency. Results of the experiment did not show any relationship between amino acids content and morphological parameters for Ko. Under nitrogen deficiency, however, root amino acids content was highly correlated to root dry weight (r=0.98), shoot dry weight (r=0.98) and total biomass (r=0.98). In addition, foliar amino acids content was correlated to shoot biomass.

We concluded from these results that Isostar, whose morphological characters (except leaf area) were severely affected, was the most sensitive. On the contrary, Flamme was the least sensitive because of its capability to maintain an adequate growth rate causing a small reduction of morphological parameters. Oro 9 had an intermediate behaviour.

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REACCION DEL GIRASOL (*Helianthus annuus* L.) A LA CARENCIA DE NITROGENO Y POTASIO

RESUMEN

El experimento fue efectuado en las condiciones de invernadero. Tres genotipos de girasol fueron investigados: Oro 9 (poblacion de variedad) y dos hibridos (Isostar y Flamme).

La carencia de nitrogeno y potasio influia considerablemente sobre los parametros morfologicos de vastago y raiz de todos los genotipos de girasol investigados. Parece que los vastagos eran mas sensibles que las raices. La reduccion de materia seca fue mas intensa en las condiciones de carencia de nitrogeno que en las condiciones de carencia de potasio.

El volumen de raiz tiene importancia en la absorcion de agua y minerales. Segun la disponibilidad normal de nitrogeno y potasio, el volumen de raiz era en alta correlacion con el peso de vastago seco ($r=0.89$), el peso de raiz seca ($r=0.84$) y el peso seco total ($r=0.86$). En las condiciones de No y Ko, la inhib-ibicon se manistaba por la reduccion del volumen de raiz y del peso de raiz seca. En las condiciones de No y Ko, el volumen de raiz era en alta correlacion con el peso de raiz seca ($r=0.99$ para No, $r=0.97$ para Ko), el peso de vastago seco ($r=0.89$ para No, $r=0.96$ para Ko) y la biomasa total ($r=-0.93$ para No, $r=0.99$ para Ko).

En las condiciones de No y Ko, la acumulacion de azucares solubles totales fue observada en todos genotipos en la fase de dos pares de hojas. La acumulacio fue en la correlacion altamente negativa con la biomasa de raiz ($r=-0.98$), en las condiciones de No. Entretanto, en las condiciones de Ko, la correlacion fue baja y negativa ($r=-0.16$). Los azucares solubles totales fueron tambien en la correlacion altamente negativa con el volumen de raiz por No ($r=-0.98$) y Ko ($r=-0.99$). Parece que los genotipos con gran contenido de azucares solubles totales, como por ej. Isostar, son mas sensibles a la carencia de nitrogeno y potasio.

La reaccion de los genotipos investigados a la carencia de nitrogeno y potasio resulto en la acumulacion de aminoacidos.

El genotipo Isostar, cuyas propiedades morfologicas (excepto la superficie de hojas) fueron a lo mas afectadas, era el mas sensible. Por otra parte, el genotipo Flamme era menos sensible a causa de la capacidad de ese genotipo de retener el crecimiento adecuado, lo que resulto en la pequeña reduccion del valor de parametros morfologicos. Oro 9 tenia valores medios.

RÉACTION DU TOURNESOL (*Helianthus annuus* L.) À LA CARENCE EN AZOTEET EN POTASSIUM

RÉSUMÉ

L'expérience a été faite dans des conditions de serre. Trois génotypes de tournesol ont été examinés; Oro 9 (population d'espèce) et deux hybrides (Isostar et Flamme).

La carence en azote et en potassium a eu une influence importante sur les paramètres morphologiques des pousses et des racines de tous les génotypes de tournesol examinés. Les pousses se sont avérées plus sensibles que les racines. La diminution de poids sec a été plus importante dans les conditions de carence en azote que dans les conditions de carence en potassium.

Le volume de la racine joue un rôle essentiel dans l'absorption de l'eau et des minéraux. Dans des conditions d'approvisionnement normales en azote et en potassium, le volume de la racine était en grande corrélation avec le poids de la pousse sèche (r=0.89), avec le poids de la racine sèche (r=0.84) et avec le poids sec total (r=0.86). Dans les conditions No et Ko, la réduction du volume de la racine et du poids sec de la racine ont montré une inhibition de la croissance causée par la carence en azote et en potassium. Le volume de la racine, sous les conditions No et Ko étaient en étroite relation avec le poids de la racine sèche (r=0.99 pour No et r=0.97 pour Ko), le poids de la pousse sèche (r=0.89 pour No et r=0.96 pour Ko).

Sous les conditions No et Ko, l'accumulation totale de sucres solubles a été observée dans deux paires de feuilles pour tous les génotypes. Sous les conditions No, l'accumulation a été en grande relation négative avec la biomasse de la racine (r=-0.98). Cependant, sous les conditions de Ko, la corrélation a été faible et négative (r=-0.16). Le total des sucres solubles a aussi été fortement et négativement en relation avec le volume de la racine pour No (r=-0.98) et Ko (r=-0.99). Il semble que les génotypes ayant un grand contenu de sucres solubles, comme par exemple l'Isostar, soient sensibles à la carence en azote et en potassium.

La réaction des génotypes à la carence en azote et en potassium a été l'accumulation d'acides aminés.

Le génotype Isostar, dont les traits morphologiques (sauf pour la surface feuillue) ont été le plus sévérement touchés, s'est révélé le plus sensible. D'autre part, le génotype Flamme s'est montré le moins sensible grâce à sa capacité de maintenir une croissance adéquate, ce qui a causé moins de diminution de la valeur des paramètres morphologiques. Oro 9 a gardé un comportement moyen.