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EFFECTS OF SODIUM NITROPRUSSIDE (SNP) ON *IN VITRO* SHOOT MULTIPLICATION OF *Agave angustifolia* HAW.

Felipe de Jesús Romo-Paz and Benjamín Rodríguez-Garay *. Unidad de Biotecnología Vegetal, Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, A.C. Camino Arenero 1227, El Bajío del Arenal, C.P. 45019, Zapopan, Jalisco, México. brodriguez@ciatej.mx

Introduction

The lack of an adequate supply of plant material for plantations, is a common problem for Agave agribusiness. Micropropagation is an efficient method to produce large quantities of clonal material in shorter periods of time than traditional methods, making efficient the continuous establishment of plantations [1]. Recently, sodium nitroprusside (SNP), a donor of nitric oxide (NO), has been used to promote regeneration of shoots in several plant species [2]. The aim of this work was to study the effect of SNP in the micropropagation of *Agave angustifolia* Haw by improving the proliferation and quality of the produced shoots.

Methods

Methods. To determine the effect of SNP, explants were placed on semi-solid MS basal medium, with the addition of L2 vitamins and supplemented with 10 mg/L benzylaminopurine (BA), 0.025 mg/L 2,4-Dichlorophenoxyacetic acid (2,4-D), 3% (w/v) sucrose and diverse concentrations of SNP (0, 10, 20, 40, 60, 100 µM). All culture media was solidified with 0.8% (w/v) agar. The media were adjusted to pH 5.8 ± 0.2 and autoclaved. Cultures were incubated at 27 ± 2°C under red light (630 nm). The experimental unit consisted of six explants in each container, and each treatment consisted of five replicates. The percentage of explants forming shoots were evaluated after 40 days of culture.

Results and discussion

100% of explants of *A. angustifolia* Haw formed buds. The multiplication of plantlets in the culture medium containing no SNP (control) was observed to be low, as the generation of new shoots per experimental unit was (25.2), in addition to short shoots (7.62 cm) as compared to other treatments ($P \leq 0.05$). The average number of shoots and shoot length was greater when the concentration of SNP increased (20 y 40 µM), finding that the addition of SNP as donor of NO positively affected the development and regeneration of *in vitro* plantlets of *Agave angustifolia* Haw (Figure 1), due to its direct effect on components of the cell wall that promote plant growth by decreasing cell-wall lignification and accelerating cell expansion [3].

Figure 1. Effect of sodium nitroprusside on shoot micropropagation of *Agave angustifolia* Haw. Bar = 5 cm.

The culture medium with 40 µM NPS, was the best statistically treatment ($P \leq 0.05$), taking into account the high number of new shoots per experimental unit (46.6) and the average length of sprouts (12.56 cm) (Figure 2).

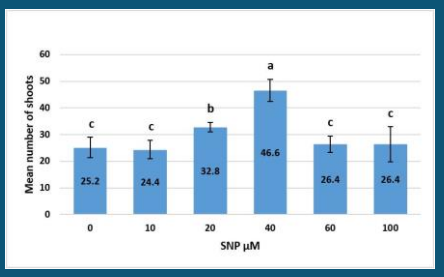

Figure 2. Effect of SNP on shoot multiplication of *Agave angustifolia* Haw. Means showing the same letter are not significantly different ($P \leq 0.05$). Vertical bars represent the standar error of the mean.

On the other hand, the multiplication of shoots decreased when the concentrations of SNP were above 40 µM. Some of the effects observed in explants treated with high concentrations of NPS (60 µM and 100 µM) was low shoot production, tissue necrosis and leaf senescence, which was probably due to increased oxidative stress that inhibited the growth of the plantlets [3], similar to that reported in *Cymbidium* sp. [4] where concluded that necrosis was due to oxidation imposed by SNP by generating a toxic H_2O_2 content. These facts demonstrate that shoot multiplication is regulated by the effect of SNP in a manner dependent on the dose, phenomenon observed in *Malus hupehensis* [5] where cell death and senescence increased as the concentration of NO increased.

Finally, the mechanisms by which NO improves efficiency in micropropagation in depth need to be elucidated. To our knowledge, this is the first report on in vitro axillary shoot multiplication of *Agave angustifolia* Haw using SNP as a nitric oxide donor.

Conclusions

The effect of SNP seems to be of a dose dependent manner and inducing *in vitro* shoot proliferation at 40µM in *Agave angustifolia* Haw.

References

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