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REGULAR ARTICLE

EXPLOITATION OF SOMACLONAL VARIABILITY FOR THE SEARCH OF SALIN-TOLERANT POTATO (*SOLANUM TUBEROSUM* L.)

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ABSTRACT

The present investigation was conducted with an aim to study the effect of salt stress (NaCl) on *in vitro* potato callus growth and the potential for exploiting this technique in improving salinity tolerance in potato. The results in the four varieties in the culture medium showed a decrease in all studied parameters like callus growth, relative average growth, water content and salinity sensitivity coefficient upon increasing the salinity of the medium in calluses. It has also been observed that the type of callus has a large effect on the above-mentioned parameters in which better callus growth adapted to 150 mmol NaCl is obtained than unsuitable calli in the same culture medium.

Keywords: Callogenesis, Potato (*Solanum tuberosum* L.), Salinity, Somaclonal Variations

INTRODUCTION

The potato (*Tuberosum solanum* L.) from the Solanaceae family is one of the most widely used crops in the world, and is the fourth most important food crop after rice, wheat and maize [1]. Sustained improvements in production will be needed to meet the needs of the rapidly growing population in a changing environment. In Algeria, the massive influx of chemical fertilizers and irrigation with brackish water constitute a major constraint for the development of the potato. Improving the tolerance of food plants to salinity, such as potatoes, becomes necessary for the exploitation of saline, natural or salt soils by local irrigation operations [2].

Genetic breeding by conventional selection of crosses to obtain salinity-tolerant genotypes is a relatively difficult approach to apply because of the time required [3]. On the other hand, *in vitro* culture selection may be a promising way to improve the tolerance of this species to salt stress, as this biotechnological approach has been used successfully in the selection of salt stress tolerant genotypes in several species.

In the field of biotechnology for crop improvement, *in vitro* culture is a key technique [4], especially for somaclonal variation, which is a phenomenon that leads to phenotypic variation of regenerated from *in vitro* culture [5, 6]. These variations can occur in isolated protoplasts or calli [7]. The purpose of this article is to study the effect of salt stress (NaCl) on *in vitro* potato callus growth and the potential for exploiting this technique in improving salinity tolerance in this potato species.

Matériel et méthodes

Our experiment took place in the SAGRODEV laboratory (Société AGRO Developement) in Sétif (Algeria). The starting plant material was supplied to us as healthy tubers by SAGRODEV. These tubers consist of four varieties namely: Désirée, Spunta Bartina and Kondor, two types of explant (leaves and internode) resulting from these varieties.

Initiation of callogenesis

The sprouts are disinfected with alcohol at 70 ° for 5 seconds, then with HgCl2 solution for 20 min and finally transplanted under sterile conditions onto a Murashige and Skoog (MS) medium [8]. Until in vitro plant are obtained.

For the induction of callogenesis, the explants (leaves and internodes taken from microplant) are cultivated in the callogenesis medium MS, containing (5 mg/L NAA+1

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mg/L BAB+1 mg/L GA3).

The pH is adjusted to 5.6-5.8 before autoclaving for 20 min at 120° C. Culture was kept for 30 d in a growth room at 25±2°C having 1.83 m fluorescent tubes and was illuminated 16 h daily with a light intensity of 1500 lux.

Adaptation of callus to salinity

The calli are adapted to the different concentrations of NaCl (00; 50; 100 and 150 g/L), added to the MS medium, supplemented with different concentrations of phytohormones (the same medium for initiation of callogenesis). The calli are cultivated under the same conditions of callogenesis.

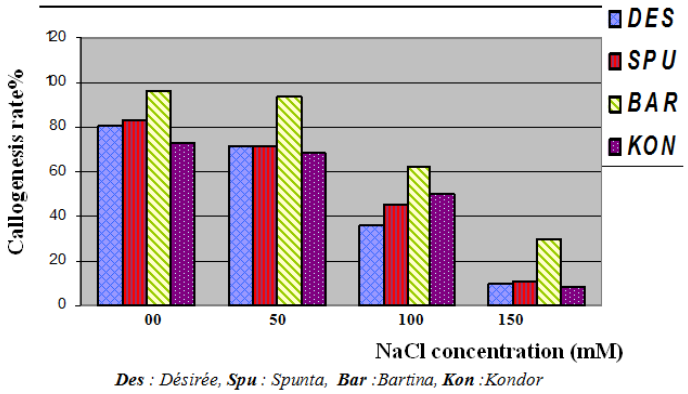
RESULTS

The callus begins to form on one side of the internode segment and then generalizes to the explant, suggesting a certain polarity of callogenesis. Fig. 1, shows that in the absence of NaCl, almost, all the explants form calluses between 72 and 95%. The presence of NaCl (50 mmol) slows the callogenesis, without much altering its final rate which is between 68 and 95%. And finally the presence of NaCl (150 mmol) considerably slows the callogenesis, which is between 10 and 30%.

In general, a decrease in callogenesis with increasing NaCl concentration in the culture medium is recorded.

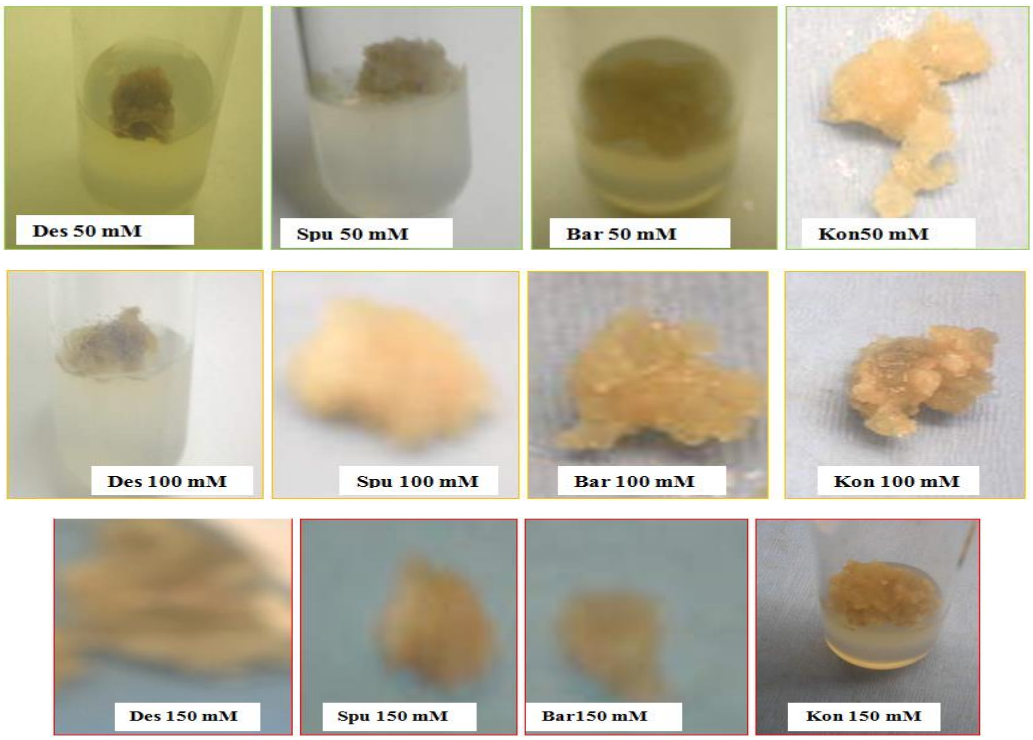
The results obtained for the type and color of the callus at the different concentrations of NaCl are shown in fig. 2. The calli are transparent and compact for the variety Desiree, friable and yellowish for Spunta but, for Bartina they are compact, green and transparent while they are compact for Kondor.

In the presence of NaCl, the calli are light green in color and have a slight brownish discolouration (fig. 2).



Des : Désirée, Spu : Spunta, Bar :Bartina, Kon :Kondor

Fig. 1: Effect of NaCl on the rate of callogenesis in four potato varieties (Désirée, Spunta, Bartina, Kondor)



Des: Désirée, Spu: Spunta, Bar: Bartina, Kon: Kondor

Fig. 2: Cals obtained after gradual selection in the saline environment for four varieties of potato: Désirée, Spunta, Bartina, Kondor

For growth of calli not adapted to salinity, at different concentrations of NaCl, the rate of decrease in fresh and dry weight is mentioned in fig. 3 and 4.

The mass of fresh and dry matter of the calli, measured after 5 w of culture, is very affected by the presence of NaCl in the medium. Indeed, calli adapted to 150 mmol salinity (SS150-200 and SS150-150) are more developed in the presence of salt (150 and 200 mmol) than those not adapted and developed in the presence of salt (CS 200, CS150) and this in the same culture medium (fig. 5). Statistical analysis shows a highly significant effect of callus type on the mass of fresh and dry matter of calli.

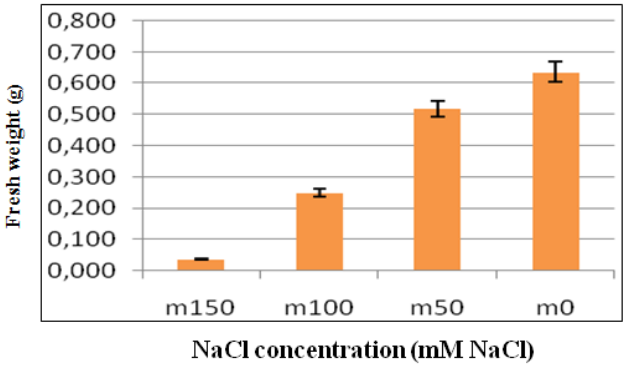


Fig. 3: Mean effect of NaCl on the fresh weight of calli in four potato varieties (Désirée, Spunta, Bartina, Kondor)

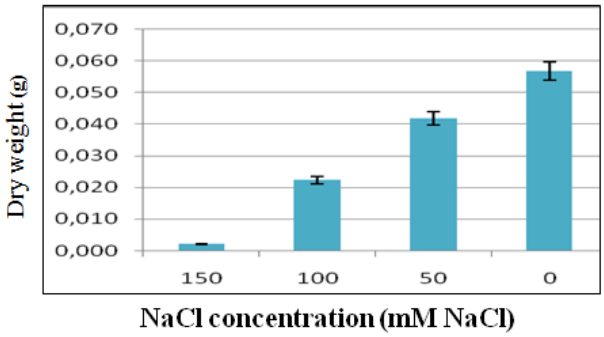


Fig. 4: Mean effect of NaCl on dry weight of calli in four potato varieties (Désirée, Spunta, Bartina, Kondor)

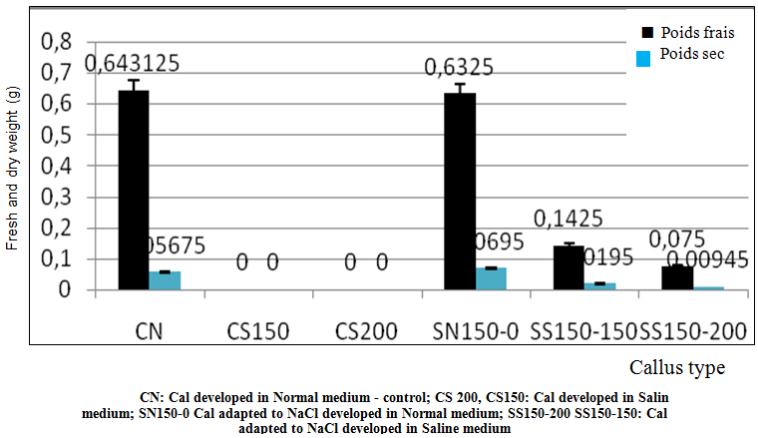


Fig. 5: Average fresh and dry weight for different types of callus in four varieties of potato (Désirée, Spunta, Bartina, Kondor)

DISCUSSION OF RESULTS

For the purpose of improving plants. The selection of plants tolerant to salinity under selective pressure, by adding a stressing agent such as NaCl, has been used for various species, more particularly, the *in vitro* culture has made it possible to select salt-tolerant variants by using calli. The callus yield is dependent on several factors,

including the nature of the explant used, the donor genotype and the culture medium used [9]. Several plants could thus be regenerated under salt stress from cell calli: wheat [10], potato [11] and Citrus [12].

Mechanisms responsible for adaptation to salinity and selection of resistant cells in laboratory conditions were investigated, however, there was not any information on the impact of salt on genetic material and creation of somaclonal variation [13]. Also, many aspects of the mechanisms that cause somaclonal variation remain undefined [6]. Part of this variation is due to gene mutations, which are transmitted sexually. These mutations do not differ essentially from those that occur spontaneously or are induced by mutagenic treatments [14]. The production of salinity-resistant calli in potatoes is largely dependent on physiological adaptation rather than causes of mutations [15].

Obtaining somaclonal variations of the salinity-resistant potato requires regeneration of plants and genetic analysis, which requires complete years. However, somaclonal variations obtained during callogenesis is an important means [16]. During the experiment, we were able to select potato calli able to grow in a culture medium supported by a high concentration of NaCl (200 mmol), with the use of *in vitro* selection; callus exposure at increasing concentrations of NaCl, with increasing concentration of NaCl by 50 mmol each. Twice, for each concentration for one month for each crop.

It can be seen from the results obtained that callus formation was observed in all the NaCl concentrations used during the experiment, given that the increase in NaCl concentration in the culture medium was accompanied by a decrease in the proportion of callogenesis, this in callus not adapted to salinity. Also the percentage decrease was different from one variety to another. These results are consistent with the results of Queiros *et al.*, [18] in potato varieties Maris, Bard and Désirée in the same concentration of NaCl [15]. The results also showed that calli obtained during potato breeding in the middle of increasing concentration of NaCl (0-50-100-150 mmol) change in color and morphology in function saline concentration.

The results obtained by Patnaik and Debata [17] and Queiros *et al.* [18] confirm that the effect of NaCl on calli is the appearance of necrosis [19]. We found that the adapted calli SS150-200 and SS150-150 are more developed than the non-saline calli CS150 and CS200 in the same concentration, which is consistent with the results of previous study [15]. We also recorded the death of all CS150 and CS200 calli (Cal developed directly in Salin medium). The results recorded for the dry weight of the calli are very similar to that of the fresh weight, and this for all varieties. High concentrations of NaCl significantly retard growth, and this is only through the low dry weight values in the 100 and 150 mmol NaCl concentrations. These results are consistent with what was obtained by Potluri and Devi Prasad [20] and Ehsanpour and Fatahian [21].

CONCLUSION

Phase control of callogenesis and adaptation to salinity by adjusting norms and optimal conditions broadens the possibility of establishing somaclonal variation based on *in vitro* culture of plant tissues, and with more efficient selection it becomes possible to produce new varieties of potatoes resistant to salinity and with better yield. In addition, the production of salinity-resistant potatoes has an economic advantage, especially in the arid and semi-arid areas of the world, the production of salinity-resistant potatoes also paves the way for development. New unconventional programs to develop the potato crop [16].

AUTHORS' CONTRIBUTIONS

Authors contributed equally to the overall study and manuscript preparation and approved the final version of the manuscript for publication.

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