



Research Article

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Determination of Saline Tolerance Under *In Vitro* Conditions of Lettuce (*Lactuca Sativa L.*) Var. Crespa Induced Polyploidy



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Abstract

Lettuce (*Lactuca sativa L.*) is a large-scale crop worldwide, which provides very few calories, high percentage of water, vitamins, minerals and fiber. But this plant species is relatively sensitive to salinity. Induction to polyploidy alters the number of chromosomes and, consequently, the proportion of allelic genes that contribute to the appearance of characters. Considering the problem generated by salinity in vegetable crops, it is intended to determine tolerance to saline stress under *in vitro* conditions of lettuce var. Crespa induced polyploidy. Lettuces were introduced *in vitro* culture, and subsequently were induced to chromosomal duplication with colchicine. It was considered to measure saline stress at different concentrations of NaCl, kept in a culture chamber under controlled temperature and photoperiod conditions for 30 days. Through the karyotype study, it was possible to determine that colchicine achieved chromosomal duplication of lettuce explants, which were initially diploid ($2n = 18$), and it was determined that polyploidy-induced explants showed greater dry weight per explant and area foliar.

Keywords: Egyptian Mongoose; *Herpestes ichneumon*; Ecology; Occurrence; Zoological Gardens; Gaza Strip

Introduction

At present, salinity is the most important environmental factor that limits the establishment and productivity of plants [1] causes countless physiological, morphological and biochemical effects in plants, such as decreased photosynthesis and quantitative and qualitative changes in Protein synthesis due to changes in gene expression due to salinity, among others [2]. Another physiological response of plants to salinity occurs by decreasing stomatal conductance; In this way, perspiration is reduced avoiding physiological drought to maintain the turgidity of the cells. The reduction of stomatal conductance implies the closure of stomata and is related, among other factors (light, humidity, CO₂, temperature and air currents), with the decrease in the potential of foliar water, even above intense light. Stomatal closure reduces the entry of CO₂ by inhibiting photosynthesis, resulting in a reduction in photosynthesis synthesis. In general, the consequence is the decrease in biomass production, such as roots, leaves, stems and seeds, related to the leaf area and the length of plants [3]. Lettuce (*Lactuca sativa L.*) belongs to the Compositae family; It is a food that contributes very few calories, high percentage of water (90-95%), vitamins (folates, pro vitamin A or beta-carotene and appreciable amounts of vitamin C), Minerals (potassium and magnesium) and

fiber. The plant is annual and autogamous, its root never exceeds 25cm. deep, is pivotal, has a short cylindrical and branched stem, the inflorescence is composed of yellow floral chapters arranged in clusters or corymbs. As for the weather, lettuce can develop at temperatures of 6°C; With a lot of difficulty. That is why cool temperate climates, with average monthly temperatures between 13°C and 18°C, allow cultivation throughout the year, although high temperatures also limit their development considerably [4]. This plant species is relatively sensitive to salinity, but such salt tolerance often varies within the same species. The threshold values for lettuce species are in the range of 1.0 to 1.4 dS m⁻¹, and the slope for the decrease in yield, from 6.2 to 8% per dS m⁻¹ [5]. Polyploidy is a natural phenomenon that occurs more frequently in plants than in animals and is considered a fundamental mechanism in the evolution of new species. About 40% of species of floral plants and between 70 and 80% of the herbs are polyploids. Many plants that are currently grown and exploited for world food are also polyploids. In the case of polyploidy induction, it is a procedure that gives the plant breeder the opportunity to modify a plant by altering the number of chromosomes and, consequently, the proportion of allelic genes that contribute to the appearance of

characters [6- 8]. Cassava plants obtained by colchicine treatment showed differences in the size of stomata when compared to the control, according to the correlation between the chromosomal number and the size of stomata observed in other plants, a group of polyploid plants of cassava [9]; thus having precedents of an alternative of detection in plants induced to polyploidy.

The use of the technique of induction of polyploidy in the improvement of plants of agricultural interest, has had promising results in much of the research with this technique, such as Matos [10] who managed to improve the production of aloe in quantities of 3 to 6 times higher compared to diploid plants. Considering the problem generated by salinity in vegetable crops, it is intended to determine tolerance to saline stress under *in vitro* conditions of lettuce var. Crespa induced polyploidy.

Materials and Methods

Table 1: Treatments for the Determination of Tolerance to Salinity.

Treatment	Ploidia	
	2n	4n
	NaCl (mM)	NaCl (mM)
T1	0	0
T2	50	50
T3	100	100

The plant material consisted of quality lettuce seeds and crespa variety obtained from a seed store in the Province of Huaura, department of Lima. Subsequently, the seeds were transferred to the facilities of the Plant Biotechnology Laboratory of the E.A.P Biology with a mention in Biotechnology for their introduction *in vitro*. First, a seed pre-disinfection process was carried out for 5 minutes with detergent and kept stirring slightly; a variant of the protocol described by Castilla y González [11] was used. In which the disinfection was carried out inside the laminar flow chamber, where it was disinfected with 70% alcohol for 1 minute and rinsed with distilled water, then it was immersed in 1.5% sodium hypochlorite for 10 minutes, it was added also a drop of tween 20, and finally rinsed 3 times with sterile distilled water. Once the seeds were disinfected, the seeds were then planted in jars with MS culture medium [12], whose pH was regulated at 5.8 and stored in the incubation chamber at 22°C regulated by photoperiods of light. In total 30 bottles were planted. When the first roots of the germinated seedlings were observed, chromosome duplication was induced, for which the seedlings were transferred to liquid media with colchicine for 24 hours. Subsequently it was performed in chromosomal analysis to determine the confirmation of duplication. Confirmed chromosomal duplication the seedlings were transferred to MS culture media, added with different concentrations of NaCl (Table 1). The variables to be evaluated were the fresh weight per seedling (FWS) and the leaf area (LA) of the seedlings after 30 days of transfer to saline stress treatments. The data obtained were studied by analysis of variance (ANOVA) and comparison test of means (Tukey, $P \leq 0.05$).

Results

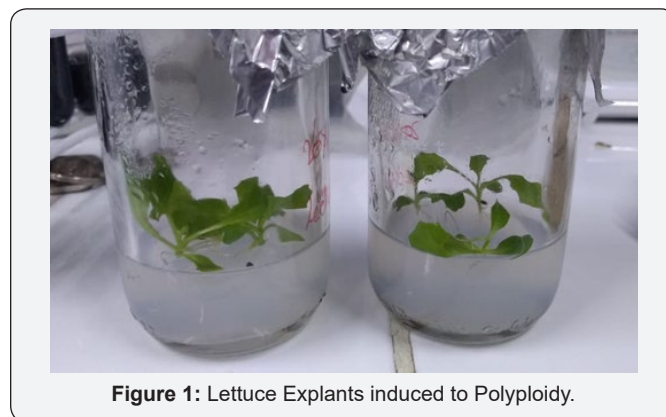


Figure 1: Lettuce Explants induced to Polyploidy.

The lettuce was germinated *in vitro* conditions without problems of contamination of bacteria or fungi. The karyotype study of the germinated explants indicated that the varieties used were diploid ($2n = 18$), while after being immersed in colchicine, variations in chromosomal structures were obtained (Figure 1). It was determined that lettuce explants that were induced to polyploidy showed greater dry weight per explant and leaf area after 30 days under conditions of saline stress *in vitro* (Table 2).

Table 2: Evaluation of the Variables under Study in different Treatments under Saline Stress.

Treatment	Ploidia			
	2n		4n	
	FWS	LA	FWS	LA
T1	0.36 b	16.4 c	0.42 a	22.6 a
T2	0.28 d	14.2 d	0.38 b	20.4 b
T3	0.21 e	8.7 e	0.34 c	15.1 c

Discussion

The karyotype study before the treatment with colchicine indicated that the seedlings were diploid ($2n = 18$), this corresponds to the statement by Archila et al. [5], that in the lettuce the genetic and chromosomal stability is maintained in conditions *in vivo* and *in vitro*. Later, after applying colchicine to the explants, variation in the chromosomal structures was obtained, obtaining results like Molero and Matos [13], who induced Aloe vera L. polyploidy through various colchicine treatments in order to induce tetraploidy in *Ex vitro* conditions from diploid plants. Both the leaf area and the fresh weight per explant expressed in Figures 2 & 3 respectively showed a decrease, since these parameters depend on cell expansion, which in turn are related to the massive cell division, but when found in a medium of osmotic difference it could affect the growth rate of the leaves [14]. In addition, the transport of sodium ions to dividing cells can affect cellular and physiological processes, as there is an increase in the concentration of sodium in the growing tissue, especially in species that have salinity sensitivity as it is the lettuce [15]. The study in the search for greater tolerance to saline stress in lettuce allows each time to use new methods of genetic improvement, which may eventually result in

high-quality plant material, exploit and take more advantage of its agri-food uses.

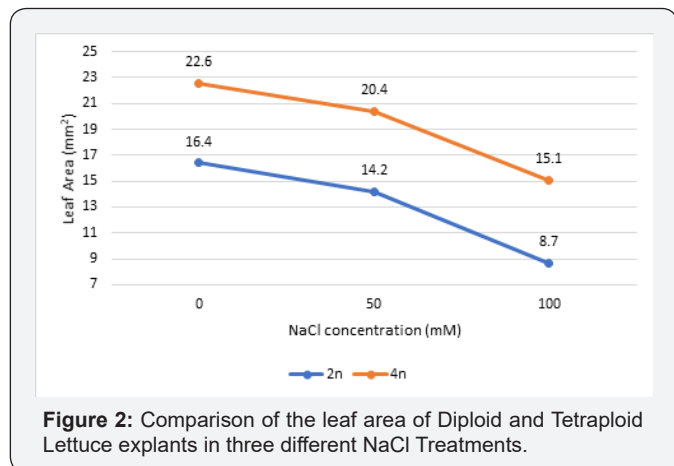


Figure 2: Comparison of the leaf area of Diploid and Tetraploid Lettuce explants in three different NaCl Treatments.

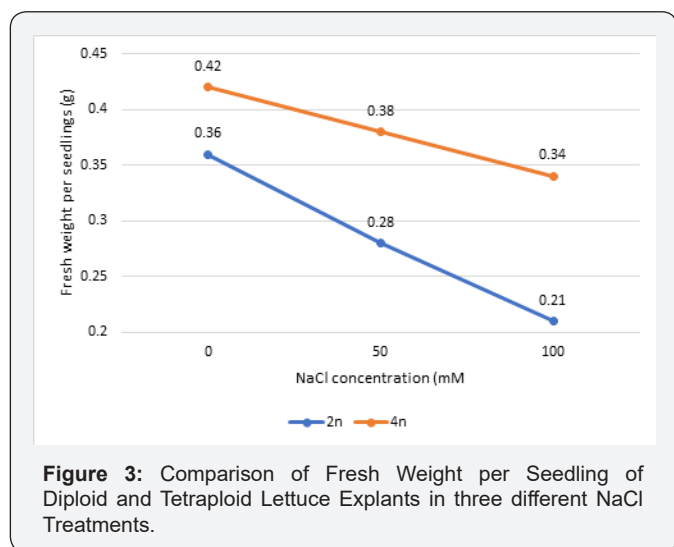


Figure 3: Comparison of Fresh Weight per Seedling of Diploid and Tetraploid Lettuce Explants in three different NaCl Treatments.

Conclusion

It was determined that the seedlings of lettuce var. Crespa induced chromosomal duplication showed greater tolerance to saline stress, because a greater foliar area and fresh weight were expressed in the explants compared to the explants that were not induced.

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