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Comparative Study for Seed Germination and Seedling Initial Growth under Elevated NaCl Concentrations to Four Species of the Brassica Genus

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ABSTRACT

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One of the most challenges that encounter humans' nutrition is the salinity increases in soil and water which leads to highly limitation in crops production. Furthermore the ability of seeds to germinate at high salt concentration in the soil is critical importance for the survival, propagation and production of many plant species. Seed germination and seedling growth of four species belong to the genus *Brassica* (*B. alba*, *B. niger*, *B. juncea* and *B. napus*) were evaluated under conditions of salinity (control, 150, 200, 250 and 300ml M). The obtained results showed that seeds of all the species germinate at all concentrations of NaCl. Percentage of viable seed germination for all tested species show slightly closely results only at the highest concentration (300 mlM) *B. napus* show significant higher seeds germination percentage than the other three species. However, root length of all the examined species slightly similar at lowest concentration while starting with concentration 200 ml MB. *napus* shows significant increase in root length than the other three tested species. *B. napus* shows more stability to the salinity condition than the other three species.

Introduction

Increasing salinity of soil is one of the most important factors of soil degradation. Osmotic and ionic effects lead to inhibition of plant growth, this is attributed to the fact that Na⁺ competes with K⁺ for binding sites essential for cellular function. So, different plant species have developed different defense responses to overcome these effects (Ines Ben Rejeb *et al.*, 2014). Salinity stress mostly reduces the germination percentage and delays the onset of germination, its effects are modified by interactions with other environmental factors as temperature

and light. Salinity can affect germination by affecting the osmotic component, which the ionic component, i.e., Na and Cl accumulation (Zivković *et al.*, 2007). Germination is the most critical period for a crop subjected to salinity (Bernstein, 1974). Seed germination has been reported to decline with increasing salinity levels (Houle *et al.*, 2001). Soil salinity in agriculture soils refers to the presence of high concentration of soluble salts in the soil moisture of the root zone. These concentrations of soluble salts through their high osmotic pressures affect plant growth

by restricting the uptake of water by the roots. Salinity can also affect plant growth because the high concentration of salts in the soil solution interferes with balanced absorption of essential nutritional ions by plants- (Tester and Devenport, 2003). Brassica seeds reported as the third largest source of edible oil following soybean and palm oil (Canola Council of Canada, 2013). This increased need will defiantly promote increased acreage of Brassica in the world where some soils are prone to become saline (Francois, 1994).

Salinity may be a problem to Brassica seedling stabilization (Tomm, 2007). Brassica seed germination (percentage and speed) decreased with increasing salinity concentration levels beyond 0.1 mol L⁻¹ using NaCl as salinity source(AL-Thabet *et al.*, 2004).This work was conducted to evaluate the effects of sodium chloride salinity levels on Brassica seed germination and seedling growth.

Materials and Methods

Conditions of Plant Cultivation and Carrying out Experiments

Work carried out on 4 species plants of genus Brassica; of spring rapeseed *B. napus* (Westar Canadian selection) *B. juncea* L, wild-type *B. niger*, and *B. alba* seeds obtained from the Baghdad region of Iraq. Seeds disinfected as follow, washed with tap water for 15 minutes then sterilized by 2 % v/v sodium hypochlorite (Clorox) for 15 minutes and then washed extensively with sterilized distilled water.

Petri dishes used with filter paper, cultivation conducted in an incubator at 25C° in dark. NaCl solution used at a concentration of 150, 200, 250 and 300 mM, with water used as control.

Measurement of Seed Germination and Seedling Root Length

Monitoring the effect of increasing NaCl concentration on germination seedling and root growth performed using at least twenty seeds in each petri dishes and each variant used three petri dishes. Disinfected seeds soaked in a solution of Sodium chloride in concentrations of 150, 200, 250 and 300 mM. Seed germination tested after 5 days, then determined the percentage of germinated seeds and measured seedlings root length. Root length of the Germinated seeds considered at least five mm. Germination of each variant considered as a percentage - the number of germinated seeds relative to the total amount of seed. All experiments performed in triple biological replicates. The results were statistically processed and expressed as the arithmetic mean and the representativeness of the error standard deviation. The experimental results statistically processed using the program Excel.

Results and Discussion

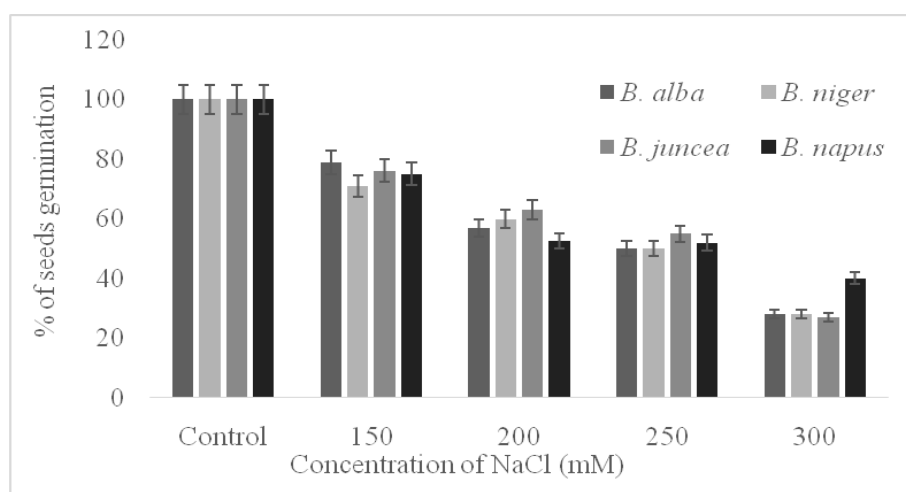
Effects of Elevated Concentrations of CuSO₄ on Seed Germination and Seedling Growth

As described in the Materials and Methods section, disinfected twenty seeds of plant laid in a petri dish on filter paper, soaked with dissolved NaCl solutions at a concentration of (150, 200, 250 and 300 mM), water served as the control. The number of germinated seeds counted and measured the length of the sprouts roots on 5thday. Viability of seed was decreased gradually with increasing NaCl concentration. At the higher concentration 300 mM NaCl *B. napus* shows more stability than the other tested Brassica species (figure 1).

Table.1 Growth of roots of seedlings Brassica species depending on the concentration of NaCl

Variant	<i>B. alba</i>		<i>B. niger</i>		<i>B. juncea</i>		<i>B. napus</i>	
	mm	%	mm	%	mm	%	Mm	%
Control	33.4	100	35.8	100	35.9	100	34.6	100
150 mM	24.1	72	27.1	76	27.1	75	27.3	79
200 mM	15.3	46	17.1	48	18.3	51	20.2	58
250 mM	7.9	24	9.1	25	11.6	32	14.8	43
300 mM	5.8	17	6.3	17	6.5	18	8.4	24

Fig.1 Percentage of seeds germination in Different NaCl Concentration



Influence of NaCl on Root Growth during Germination of Brassica Seeds

Elevated concentrations of Sodium chloride had a negative effect on seed root of Brassica, highly loss of germination occurred at 300 mM NaCl. Brassica greater stability clearly visible at 150 mM NaCl (table 1). Further evaluation of the effect of copper on the growth of the seedlings roots. Elevated concentrations of NaCl significantly reduces the length of seedling roots of the four tested Brassica species (Table 1). Significant decrease in growth began with 150mM NaCl and at the highest concentration (300 mMNaCl) used, root growth decreased to 70 % of control except for *B. napus* which decreased about 60 % of

the control. These results suggest that in the initial stages of plant Brassica showed fairly acceptable tolerance to high concentrations of NaCl.

Different Plants shows broad diversity in response to soil salinity tolerance. Plant adaptation or tolerance to salinity stress involves complex physiological traits, metabolic pathways, and molecular or gene networks (Bhaskar Gupta and Bingru Huang, 2014). Continuous water evaporation causes surface salt deposition (Ungar, 1991), while rain dissolves and washes away salt deposits and provides enough water for germination. In the course of evolution, seeds have adapted to such changes, staying viable at high soil salinity

and being able to germinate under appropriate external conditions (Khan and Ungar, 1997). Reduction in seed water uptake caused by changes in osmotic potential and causes negative effects on seed germination, considering that water is essential for seed storage reserves digestion and for all germination phases (Bewley *et al.*, 2013). Furthermore, induction of primary effects like ionic stress which in turn induce oxidative stress in plants resulted from salinity (Ahmad *et al.*, 2012). Reactive oxygen species (ROS) is highly deleterious for plants generated as a result of oxidative stress. ROS can destroy the structure and functions of biomolecules such as membrane lipids, proteins and nucleic acids (Hernandez *et al.*, 2001; Ahmad *et al.*, 2010) and causes death of the plant cells in higher concentrations, (Ahmad and Sharma, 2008). Also, salinity causes ion cytotoxicity by the replacement of K⁺ by Na⁺ in biochemical reactions and by the loss of function of proteins, as Na⁺ and Cl⁻ ions penetrate the hydration shells and interfere with the non-covalent interaction among the amino acids (Zhu, 2002). The groups of plants that are well adapted to saline habitats are called halophytes. Their seeds germinate well in freshwater and the germination is similar to that of seeds of non-adapted species. However, they differ from them in ability to germinate at higher salt concentrations in the soil. Salinity tolerance of many perennial halophytes dependent on a variety of abiotic factors (Baskin and Baskin, 1998). This results showed that seeds of all tested species of Brassica germinated at low concentrations of NaCl (150 mM), and with higher concentrations (300 mM) seeds these species were reduced with *B. napus* as the higher tolerance. Although species from families Brassicaceae are not typical halophytes, their seeds germinated well at a moderately elevated salt concentration. Published data show that the

effect of NaCl on seed germination is mostly osmotic (Loercher, 1974; Reynolds, 1975), but the nature of its effect is unknown. The effect of salts on plants leads to physiological drought. Salt reduces the water potential of soil solution, which prevents the supply of water by plants. They in the salty soils receiving large amounts of salt in root cells, and thus reduces the water, so it increases the absorption of water in physiological drought conditions (Silva and Talhaferro, 2014). In this study all tested species of Brassica shows similar responses to the increasing concentration of NaCl except *B. napus* at the higher concentration of NaCl (300 mM) slightly tolerate NaCl more than the other tested species which may be considered as moderately halophytes.

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