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Screening of Salt Tolerance Canola Cultivars (*Brassica napus* L.) Using Physiological Markers

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Abstract: In order to identification of salt tolerance genotype, an experiment with seven rapeseed cultivars under different levels of salt stress treatments (0,7 and 14 dsm^{-1}) has been undertaken using a factorial experiment based on completely randomized design with four replications in greenhouse at Sari Agricultural Science and Natural Resources University (SANRU). During vegetative growth, shoot Na^+ and K^+ contents, K^+/Na^+ ratio and grain yield corresponding with salinity susceptibility index (SSI) and salt tolerance index (STI) were measured. In general, tolerance cultivars (LSG2 and LSN) with higher agronomic performance, contained lower Na^+ and higher K^+ and K^+/Na^+ ratio than in tolerance cultivars (Sarigol and RGS003). Shoot Na^+ contents showed negative correlation with grain yield ($r = -0.384$, $p<0.05$) and STI ($r = -0.392$, $p<0.01$) but positive correlation with SSI($r = 0.406$, $p<0.01$). These two indices have been considered as useful criteria for screening of tolerance cultivars under salt stress.

Key word: CANOLA cultivars screening · Salt tolerance · K^+ · Na^+ · STI · SSI

INTRODUCTION

Soil salinity is one of the major environmental stresses affecting plant growth and productivity [1]. Salinity induces water deficit even in well watered soils by decreasing the osmotic potential of soil solutes thus making it difficult for roots to extract water from their surrounding media [2]. The effect of high salinity on plant can be observed at the whole plant level in terms of plant death and/or decrease in productivity [3]. Crop yield start declining when pH of the soil solution exceeds 8.5 or EC value goes above 4 $d\,S\,m^{-1}$. At higher EC values the crop yield are reduced so drastically that crop cultivation is not economical without soil amendments [4]. Salinity stress biology and plant responses to high salinity have been discussed over two decades [5-8].

The varietals differences in salinity tolerance that exist among crop plants can be utilized through screening programs by exploiting appropriate traits for salt tolerance; Grain yield is frequently used in crops such as rapeseed as the main criterion for salt tolerance [9]. Also the use of physiological markers such as content of Na^+, K^+ and the ratio of K^+: Na^+ are less feasible and in view of some researchers are not promising [10]. However, it is believed that selection and breeding would be more successful in achieving maximum attainable tolerance, if it were based directly on the relevant agronomic and physiological mechanisms [11].

Salt stress result in a considerable decrease in the fresh and dry weights of leaves, stems and roots [12]. High ionic concentration competes with the uptake of other nutrients, especially K^+, leading to K^+ deficiency. Increasing NaCl can increase Na^+ and Cl^-ions and decrease Ca^{2+}, Mg^{2+} and K^+ ions in a number of plants [7]. There is a negative relationship between Na^+ and K^+ concentrations in roots and leaves. The selective uptake of K^+ as opposed to Na^+ is considered to be an important physiological mechanism contributing to salt tolerance in many plant species [6, 13]. It is well documented that a greater degree of salt tolerance in plant is associated with a more efficient system for selective uptake of K^+ over Na^+ ions [14, 11]. It has been reported that salt tolerant barley

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varieties maintained lower Na^+ than non-tolerant ones [15]. Plant ionic uptake status along with agronomic traits and their relationships with salt tolerance indices have been considered as useful selection tools for screening of salt tolerant [1]. In present study, leaf Na^+ and K^+ contents along with some agronomic and physiological traits were evaluated for salt tolerance in seven rapeseed cultivars.

MATERIALS AND METHODS

Plant Materials: Seven rapeseed cultivars (LSG1, LSG2, LSN, RGS003, KTL2, Hyola401 and Sarigol) were compared at 3 salinity levels [0 (control), 7 and 14 dSm^{-1}] for measuring their Na^+, K^+, K^+: Na^+, grain yield and two salinity indices [salinity susceptibility index (SSI) and salt tolerance index (STI)], in a factorial experiment based on a completely randomized design with 4 replications. The experiment was conducted in greenhouse at Sari Agricultural Science and Natural Resources University (SANRU). Ten seeds were initially planted in each 12 kg pots filled by clay loam soil (35% clay, 27% sand and 38% silt, PH 7.36 and EC 0.73). The Plants were watered according to field capacity using tap water. All but four plants were removed at four leaf stage in each pot.

Salt Treatments Application: The plants were subjected to three treatments: no salt (control), 7 and 14 dSm^{-1} [5 g salt (NaCl and Na2SO4 in 1:1 ratio) per kg soil]. Salt stress treatments were applied 6 weeks after planting (at 4 leaf stage).

Na^+, K^+ and K^+: Na^+ Ratio Measurements: Six weeks after applying salt treatments, leaf samples were collected, washed in distilled water to remove any external salt and dried at 60°C oven for 48 h. Dried samples were ground into a fine powder using a mortar and pestle. Samples (1 g) were ashes by putting them into crucibles and placed in 600°C electric furnace, for 4 h, 5 mL of 2 N HCl were added to cooled ash samples, dissolved in boiling deionized water, filtered and made final volume to 50 mL. Na^+ and K^+ were measured using standard flame photometer procedure [16] and reported as mg g^{-1} dry weight.

Agronomic Traits Measurement: The stress susceptibility index (SSI) was calculated for grain yield of each cultivar using following formula [17]:

$$SSI=(GY_s/GY_p)/1\text{-}D$$

Where, GYs and GYp are means yield of cultivar under salt stress and non-stress (control) conditions, respectively. D is the ratio of overall mean of cultivars under stress on overall mean of cultivars on control. Salt tolerance index (STI) was calculated for grain yield of each cultivar as:

$$STI=Y_s/Y_c$$

Where, Ys and Yc are means of cultivar under salt stress and control condition, respectively.

Statistical Analysis: Data were analyzed using MSTATC statistical software package and Pearson correlation analysis was performed using SAS statistical software package.

RESULTS AND DISCUSSION

Analysis of variance and significant differences of Na^+, K^+, K^+: Na^+ and agronomic traits for salinity and cultivar factors are presented in Table 1. Na^+, K^+, K^+: Na^+ and grain yield were highly significant ($p< 0.01$) for salinity and cultivar factors.

Na^+, K^+ contents and K^+: Na^+ ratio: Salt treatments (0, 7 and 14 dSm^{-1}) had significant effects on Na^+, K^+ uptake and K^+: Na^+ ratio of rapeseed cultivars (Table 1). Rapeseed cultivars were different in regard to Na^+, K^+ content and K^+: Na^+ ratio EC= 14 (Table 2). This indicates the existence of genetic diversity for these traits among the rapeseed cultivars.

"LSG2" (a salt tolerant cultivar) had the lowest Na^+ content and the highest K^+: Na^+ ratio, whilst "Sarigol" (a salt sensitive cultivar) had the highest Na^+ content and the lowest K^+: Na^+ ratio. Cultivars "LSN", "KTL2" and "LSG1" having lower Na^+ and higher K^+: Na^+ ratios may considered as salt tolerant cultivars and the cultivars "Sarigol" and "RGS003" with higher Na^+ content and lower K^+: Na^+ ratios may considered as non-tolerant cultivars (Table 2).

Agronomic Traits: Cultivars were different regarding their grain yield at higher salt level (Table 2). The highest grain yield was given by "LSG2" and the lowest was belonged to "Sarigol". Correlation between grain yield and Na^+ was negative ($r=-0.384$, $p< 0.05$, Table 3).

Table 1: Analysis of variance for traits investigated in 7 rapeseed cultivars in response to salinity stress

Source	df	Na^+	K^+	K^+/Na^+	Grain yield(g)
Salinity	2	3.575^{**}	7.016^{**}	94.95^{**}	14.19^{**}
Cultivar	6	1.061^{**}	3.311^{**}	25.1^{**}	7.73^{**}
Salinity * Cultivar	12	0.188^{**}	0.195^{*}	3.31^{**}	1.06^{**}
error	60	0.062	0.161	0.994	0.249
Cv(%)		29.69	21.51	32.09	15.31

* and ** Significantly at $p < 0.05$and < 0.01, respectively

Table 2: Average of evaluated criteria at EC = 14 dS m^{-1} in 7 rapeseed cultivars

Cultivar	Shoot Na^+(mg g^{-1})	Shoot K^+(mg g^{-1})	K^+/Na^+(mg g^{-1})	Grainyield (g)	STI	SSI
LSG1	1.02^{c}	1.51^{b}	1.05^{bc}	3.11^{c}	0.81	0.53
LSG2	0.71^{c}	2.05^{a}	3.27^{a}	3.68^{a}	0.91	0.26
LSN	0.74^{c}	1.15^{b}	1.63^{b}	3.25^{b}	0.87	0.37
RGS003	1.58^{b}	1.11^{b}	1.09^{bc}	1.62^{de}	0.45	1.55
KTL2	0.86^{c}	1.56^{b}	0.79^{bc}	1.80^{d}	0.39	1.66
Hyola401	1.60^{b}	1.47^{b}	0.97^{bc}	3.59^{a}	0.65	0.99
Sarigol	2.09^{a}	0.53^{c}	0.617^{c}	1.26^{e}	0.41	1.77
$LSD_{5\%}$	0.207	0.326	0.5299	0.964		

Means followed by same letter (s) in each column are not significantly different ($p < 0.05$)

Table 3: Correlation coefficients between Na+, K+, K+/Na+, agronomic traits and salinity indices of 7 rapeseed cultivars

	Na	K	K/Na	yield	STI	SSI
Na	1					
K	$^{**}0.551$	1				
K/Na	-0.526^{*}	0.676^{**}	1			
yield	-0.384^{*}	0.669^{**}	0.583^{**}	1		
STI	-0.392^{*}	0.551^{**}	0.721^{**}	0.862^{**}	1	
SSI	0.406^{*}	-0.534^{*}	-0.716^{**}	-0.888^{**}	0.999^{**}	1

* and ** significantly difference at $p<0.05$ and $p<0.01$ respectively

Therefore, salt tolerant cultivars having lower Na^+ content, produced higher grain yield under saline conditions. Akram *et al.* [18] showed that salinity reduces yield primarily by a sever reduction in grain number, 1000 grain weight and the grain yield. Same results were obtained for barley genotypes under saline conditions [15].

Salinity Indices: There were variations between rapeseed cultivars in regard to SSI and STI under saline conditions (Table 2). "LSG2" had the lowest SSI and the highest amount of STI. In contrast, "Sarigol" had the highest SSI and the lowest of STI values. There was significantly positive correlation between Na^+ and SSI ($r= 0.406$, $p< 0.01$) showing that, non-tolerant cultivars having higher Na^+ contents, also have higher SSI index. Correlation between Na^+ and STI was negative ($r=-0.392$, $p< 0.01$). Positive correlation of SSI with Na^+ and negative one of STI with Na^+ revealed that by increasing $Na^{+.}$ there would be an increase and a decrease in SSI and STI, respectively (Table 3). Results indicated that physiological traits (Na^+ and K^+: Na^+ ratio) and salinity indices (SSI and STI) are suitable indices for screening salt tolerant cultivars because of their strongly correlations with grain yield in rapeseed.

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