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Soluble proteins, a biochemical indicator for salinity screening in wheat cultivars (*Triticum aestivum* L.)

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ABSTRACT

Soluble protein concentration of seventeen wheat cultivars (*Triticum aestivum* L.) in NaCl salinity (16 dS m^{-1} and two weeks exposure) was assessed to evaluate the ability of these traits in salt screening. There was an increase in protein content in salinity stress compared to control condition in root and shoot. Even thought, there was a clear relation between salt tolerance or salt sensitivity and soluble protein in shoot plant; we didn't find any relation between tolerance and protein concentration in root. It seems that the increase in concentration of soluble protein can be a criteria to distinct salt tolerance in the shoot of early wheat plant. Therefore soluble protein concentration measurement can be applied with other trait such as Na exclusion, yield and yield components to screen wheat cultivars in salinity condition.

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Introduction

Soil salinity is a considerable problem adversely affecting physiological and metabolic processes, significantly reducing growth and yield (Cuartero et al. 2006). Plants response in two separate phases towards salinity stress: In the first, osmotic phase, which is the reduce of plant root ability to water uptake and falling shoot growth. In this phase leaves expansion ratio, new leaves emergence and lateral buds development would be slow. The second, ion toxicity phase, when concentration of ions (mostly Na^+ and Cl^-) increases to a certain threshold level, plant cell cannot storage this ion concentration consequently cell begins to die. In this condition solutes injure intercellular organism, plasma membrane and enzymes activity(Munns and Tester 2008).

Improving crop salt tolerance and pasture species require access to new genetic diversity (either natural or transgenic), and efficient techniques for identifying salt tolerance. In international collections there may be a wide range of genetic diversity in salinity tolerance that is undiscovered and unconsidered. Varity introduction is the easiest and the most economical strategy to improve stress tolerance and yield. To this purpose, first we require a screening method to identify cultivars stress tolerance. Munns and James (2003) recommend three basic methods for salinity tolerance screening. First, screening methods based on growth or yield, that is classic approach in most studies. This method requires long term experiment and is very expensive. Second, screening methods based on damage or tolerance to very high salinity levels, that evaluate large number of genotype for germination and survival parameters in high level salinity. Using injuries or survivals to identify salt-tolerant germplasm arises limitations when the cause of injury is not known. Salinity tolerance is a plant complex response including many physiological, morphological and phonological processes. Ashraf and Harris (2003) named some of these traits, biochemical indicators for salinity tolerance in plants. Therefore the third method is based on physiological mechanisms.

Some researchers (Flowers and Yeo 1995, munns and Jamse 2003, Ashraf and Harris 2003) have suggested that screening for salt tolerance being carried out using physiological markers, or that physiological traits should be used as selection criteria, either singly or in combination, rather than selection being simply applied upon yield or yield components. There is a great deal of researches that show the importance of water relations, photosynthesis, and accumulation of various inorganic ions and organic metabolites in salinity tolerance. Some of these studies report that the acclimation of proline(Chen et al., 2009), soluble proteins (Goudarzi and Pakniyat 2009), glycine-betain(Kathuria et al., 2009) antioxidant enzymes (Goudarzi and Pakniyat 2009) and some of soluble sugers such as trehalose (Suárez et al., 2009) and mannitol (Maheswari et al.,2010) can be indicators for salinity tolerance screening. In the other hand, selection in the field is not efficient because soil salinity varies substantially with time, location, soil type, and depth. Furthermore, it has been reported that little relationship exists between tolerance at germination and later growth stages in many crops. Therefore, most of recommended screening methods are based on greenhouse experiments, in this condition yield and biomass don't have a clear relationship with yield or biomass in field condition so the study of physiological traits can be a beneficial method in greenhouse.

Proteins are the most important class of biochemical macromolecules; they have structural and functional roles in cell processes. Changes in protein expression, accumulation, and synthesis have been observed in many plant species as a result of plant exposure to stress during growth. Both quantitative and qualitative changes to proteins were detected during stresses. Several salt-induced proteins have been identified in plants species and have been classified into two distinct groups. salt stress proteins, which accumulate only due to salt stress, and

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stress associated proteins, which also accumulate in response to heat, cold, drought, water logging, and high and low mineral nutrients. Osmotin and osmotin-like are the best examples for stress induced proteins, osmotin is a stress-responsive multifunctional 24-kDa protein and provides osmotolerance to plants. Osmotin, an antifungal cytotoxin, is also a plant pathogenesis-related protein that causes rapid cell death in the yeast (*Saccharomyces cerevisiae*; Kupchak et al. 2008). While investigating the mechanisms of salt tolerance in a mangrove (*Bruguiera sexangula*) Yamada et al. (2002) found a specific protein, allene oxide cyclase (AOC) responsible for enhanced salt tolerance. They designated this protein as "mangrin". Furthermore, expression of mangrin in Saccharomyces cerevisiae and tobacco cell lines also enhanced salt tolerance in these species. In this study, we attempt to assess total soluble protein measurement that may provide a quick and reliable screening test on wheat early plant that will save time and cost.

Material and methods

This study was carried out in greenhouse from the middle of October to the middle of November 2010. The air temperature ranged from 22 to 28 ˚C during the day and 14 to 17 ˚C during the night. The experimental treatments were arranged as factorial based on a completely randomized design with three replications. Treatments consisted of two levels of salinity [1.6(control), and 16 dS m^{-1}(salinity stress)] and wheat cultivars. Seventeen cultivars of spring wheat (*Triticum aestivum* L.) from Iranian economic cultivars were used in this study. They were Roshan, Tabasi, Hirmand, Chamran(as tolerance cultivars), Atrak, Tajan, Ghods, Shiraz(as sensitive cultivars), Falat, Alvand, Kavir, Mahdavi, Niknejad, Dez, Pishtaz and Star(as intermediate cultivars).

3.5 kg of soil (mixture of farm soil, sand, and farmyard manure in a 3:2:1 ratio) were put into the pots. Ten grains were sowed in each pot. They were daily irrigated with water until seedlings establishment (21days). Afterwards, the pots were watered to the saline levels (16 dS m^{-1}) by adjustment the water content of soil nearby the field capacity. After 14 days (35 days after sowing) shoot and root of plant was separated manually and frozen by liquid nitrogen and keep in -70°C freezer until determination of soluble proteins.

Total soluble protein content was measured according to Bradford (1976) using bovine serum albumin (BSA) as a protein standard. Fresh leaf samples (1 g) were homogenized with 4 ml Na-Phosphate buffer (pH 7.2) and then centrifuged at 4°C. Supernatants and dye were pipetting in spectrophotometer cuvettes and absorbances were measured using a Uv-vis spectrophotometer (shimadzu, UV-160) at 595 nm.

Data were subjected to a factorial based on a completely randomized design with three replications and the analysis of variance of the data was done by SAS, 9.1software. The means were separated by Duncan's multiple range test (DMRT) at the 5% probability level.

Results and Discussion

As the analysis of variance in the data showed, soluble protein in this experiment was significantly affected by salinity. The wheat cultivars utilized also responded differently to salinity stress ($p<0.01$, data not showed). Mean comparisons between the tissues showed that each tissue, shoot and root, increases soluble protein in salinity condition. Even thought the increase in both of them was the same (around 13%), soluble protein concentration was significantly higher in shoot tissue compared to root tissue. soluble protein concentration in shoot was 4-fold higher than root (Table 1). In response to salinity, plants make new proteins that help them to grow and develop under saline condition. One may speculate that, salt tolerant cultivars producing higher protein concentration is due to higher efficiency of osmotic regulation mechanism in these plants which in turn causes decreasing sodium toxicity in cytoplasm compared to susceptible ones and the result is to prevent proteins reduction under salt stress (Flowers and Yeo, 1995).

An increase in soluble protein concentration of salt tolerant wheat cultivars (Roshan, Tabasi, Hirmand, Chamran) has been showed during salinity stress in shoot and as well as a decrease in salt sensitive cultivars (Atrak, Tajan, Ghods, Shiraz). The highest and lowest change in soluble protein concentration was observed at Hirmand and Niknejad, respectively(Table 2). Salt-induced increase in total soluble proteins was earlier reported by Amini and Ehsanpour (2005) in 21-day-old tomato plants and by Afzal et al. (2006) in wheat seedlings. The increase in total soluble proteins may have been due to the synthesis of osmotin like proteins or structural proteins (Amini and Ehsanpour, 2005). Even thought in this study we observed a reduction in protein content in salt sensitive cultivars, that didn't support with other studies, it can be a hopeful result to distinct salt tolerance and salt sensitivity in this salinity condition (16 dS m-1 and two weeks exposure).

In contrast, there wasn't any clear relation between each salt tolerant or sensitive cultivars in salinity condition with protein concentration. For example, protein concentration increased in Hirmand (salt tolerant) and Shiraz (salt sensitive) or decreased in Tabasi (salt tolerant) and Ghods (salt sensitive) (Table 2). The large number of studies have been showed that (reviewed by Main et al 2011), excluder plants (such as wheat) to reduce ion toxicity, accumulate Na^+ ions in root and inhibit Na^+ translocation to shoot tissue and specifically to leaves. This may be a reason for different responses of root in salinity condition compared to shoot. In other words, because of high concentration of Na^+ in root and the difference of cultivars to store Na^+ in root, there isn't a clear response of soluble protein concentration in salt tolerant and sensitive cultivars.

An appropriate trait for screening should have two important features; first it should be able to distinct salt tolerant or sensitive cultivars between a certain number of cultivars. Second, these measurements should be possible in early plant growth phase. Our results, indicated soluble protein concentration in early wheat shoot, could be a suitable trait for screening in salinity stress. However it seems that the usage of an integrated method including Na^+ exclusion, yield and yield component can be most effective.

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Table 1. Mean comparison for soluble protein concentration in root and shoot

Tissues	Treatments		
Shoot	Control	37.36	B
Shoot	Salinized	42.28	A
Root	Control	8.548	D
Root	Salinized	9.822	C

Table 2. Mean comparison for soluble protein concentration in 17 wheat variety

	Shoot				Root		
variety	normal	salinity	change%		normal	salinity	change%
Alvand	32.06	36.7	14.5		9.003	11.61	29.0
Atrak	48.33	41.49	-14.2		7.1	12.17	71.4
Chamran	39.22	48.25	23.0		8.413	9.033	7.4
Dez	36.5	43.4	18.9		6.477	11.31	74.6
Flalat	34.82	42.25	21.3		5.75	12.57	118.6
Ghods	35.49	30.07	-15.3		10.97	6.9	-37.1
Hirmand	34.31	49.01	42.8		4.77	9.96	108.8
Kavir	41.77	40.9	-2.1		7.38	9.733	31.9
Mahdavi	35.46	48.62	37.1		9.37	11.59	23.7
Marvdasht	27.69	34.67	25.2		10.02	9.677	-3.4
Niknejad	41.88	35.37	-15.5		9.567	7.267	-24.0
Pishtaz	41.18	50.72	23.2		10.07	6.283	-37.6
Roshan	43.9	51.64	17.6		9.68	8.67	-10.4
Shiraz	33.52	38.6	15.2		8.893	11.53	29.7
Star	33.55	40.82	21.7		8.08	10.32	27.7
Tabasi	42.16	54	28.1		10.07	8.81	-12.5
Tajan	33.24	32.23	-3.0		9.707	9.54	-1.7
LSD	2.1	2.4	-		0.9	1.2	-