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Effects of salinity and salinity-induced augmented bioactive compounds in purslane (*Portulaca oleracea* L.) for possible economical use

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ABSTRACT

Dry matter (DM), total phenolics, flavonoids, carotenoid contents, and antioxidant activity of 12 purslane accessions were investigated against five levels of salinity (0, 8, 16, 24 and 32 dS m^{-1}). In untreated plants, the DM contents ranged between 8.0–23.4 g/pot; total phenolics contents (TPC) between 0.96–9.12 mg GAE g^{-1} DW; total flavonoid contents (TFC) between 0.15–1.44 mg RE g^{-1} DW; and total carotenoid contents (TCC) between 0.52 BCE g^{-1} DW. While FRAP activity ranged from 8.64–104.21 mg TE g^{-1} DW (about 12-fold) and DPPH activity between 2.50–3.30 mg mL^{-1} IC_{50} value. Different levels of salinity treatment resulted in 8–35% increases in TPC; about 35% increase in TFC; and 18–35% increases in FRAP activity. Purslane accessions Ac4, Ac5, Ac6 and Ac8 possessed potentials for salinity-induced augmented production of bioactive compounds which in turn can be harnessed for possible human health benefits.

1. Introduction

Soil salinity is a major threat to global food security. Up to 20% of the world's irrigated land, which produces one third of the world's food, is salt affected (Abogadallah, 2010). Salt stress causes a range of adverse effects in plants. First, it imposes osmotic stress by reducing the soil water potential leading to limiting the water uptake. Second, it causes excessive uptake of ions particularly Na^+ and Cl^- resulting in nutritional imbalances and ultimately interfering with various metabolic processes (Munns & Tester, 2008). A common feature of these effects is to trigger overproduction of reactive oxygen species (ROS) and causing oxidative stress (Hu, Li, Zhang, Luo, & Fu, 2011). Under normal growth conditions, the ROS molecules are scavenged by various antioxidative defense mechanisms and equilibrium prevails between the production and the scavenging of ROS (Foyer & Noctor, 2005). This equilibrium may be perturbed by various biotic and abiotic stresses including salinity leading to sudden increase in intracellular levels of ROS. ROS are extremely reactive in nature and can interact with a number of other molecules and metabolites such as DNA, pigments, proteins, lipids, and other essential cellular molecules which lead to a series of destructive processes in plants (also in animals) and at severe stress conditions, leads to plant death (Gill & Tuleja, 2010).

In response to salt stress (also to other stresses), plants have evolved varying degrees of adaptation processes. The essential processes leading to plant adaption to salt stress include control of water loss through stomata, metabolic adjustment, toxic ion homeostasis, and osmotic adjustment (Munns & Tester, 2008). Plant responses to the osmotic and ionic components of salt stress are complicated and involve many gene networks and metabolic processes. Such responses depend mainly on the inherent salt tolerance of the plant, the severity of salt stress and the duration of exposure of the plant roots to the salt (Munns & Tester, 2008).

However, to detoxify harmful erects of ROS from damaging cellular components; plant cells are equipped with excellent antioxidant detoxification mechanisms. The antioxidant defenses could be either non-enzymatic through different phenolic compounds and carotenoids (e.g. glutathione, proline, α-tocopherols, carotenoids and flavonoids) or enzymatic (e.g. superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) (Gill & Tuleja, 2010). The significance of detoxification of ROS though was a matter of debate in recent past, a great deal of researches have established that the induction of the cellular antioxidant machinery is important for protection against various stresses (Gill & Tuleja, 2010; Petridis, Therios, Samouris, & Tananaki, 2012). The correlation between antioxidant capacity and salt tolerance have been

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demonstrated in a large number of plants, including salt-tolerant glycophytes and true halophytes such as *Beta maritime*, *Cassia angustifolia* and *Crithmum maritimum* (Li, 2008). It has also been proved as an essential component of salt tolerance based on studies on mutant and transgenic plants with enhanced capacities to scavenge ROS that showed higher salt tolerance (Gill & Tuleja, 2010).

Further, plant materials containing high phenolic compounds and carotenoids are increasingly of interest for the food industry as they participate in the defense against ROS. Thus, salinity-stressed plants might represent potential sources of bioactive compounds for economical use. Population-based epidemiological studies also have stressed the important role of diet and lifestyle in the emergence of many degenerative chronic diseases such as cancers and cardiovascular diseases, in both developed and developing countries (Dehghan et al., 2012). If a global health goal is to increase the amounts of vitamins, minerals or bioactive compounds consumed in the diet, then a sensible approach is to further enhance the nutritional content of regularly consumed foods such as vegetables.

Purslane (*Portulaca oleracea*) is an herbaceous annual in the family Portulacaceae, found in most corners of the globe and traditionally has been treated as a major weed of vegetables and other crops. However, researchers and nutritionists have proved this plant as a potential vegetable crop for human consumption due to its nutritional and pharmaceutical importance. It is one of the richest vegetable sources of omega-3 fatty acids, α-linolenic acid, α-tocopherol, ascorbic acid, β-carotene and glutathione (Alam et al., 2014; Simopoulos, Norman, Gillaspy, & Duke, 1992; Uddin, Juraimi, Ali, & Ismail, 2012). The lack of vegetable sources of omega-3 fatty acids has resulted in growing interests to introduce purslane as a cultivated vegetable. More importantly, purslane has been reported with high antioxidant properties mainly due to its high phenolic compounds contents compared to other common vegetables. However, greater biosynthesis of phenolics is often induced when plants are exposed to environmental stresses, such as salinity. Purslane is highly adaptable to various stress environments and this characteristic gives purslane competitive advantages over many other cultivated crops. Purslane has been proved to be more salt-tolerant than any other vegetable crop (Yazici, Turkan, Sekmen, & Demiral, 2007) and can produce enough biomass under moderate salinity stress which other vegetable crops cannot (Kafi & Rahimi, 2011). Therefore, purslane could be a novel plant to be grown in moderately saline soils to potentially augment its production of bioactive components which in turn probably can be harnessed for human health benefits.

Much information is amiable on the mineral nutrient contents of purslane and their changes due to salinity stress. However; information are scanty on the bioactive compounds of purslane and impacts of salinity stress on them. In this study, total phenolics, flavonoids, carotenoid contents and antioxidant properties have been investigated in untreated control and salt-treated purslane plants. The other objective was to investigate whether salinity-stressed purslane plants can represent potential sources of bioactive compounds for economical use.

2. Materials and methods

2.1. Purslane accessions and study location

The 12 purslane accessions used in this study were collected from different locations of West Peninsular Malaysia. Among those, 10 were ornamental purslane (Ac1-Ac10) and two were common purslane (Ac11 and Ac12). The experiment was conducted in a glasshouse at the Faculty of Agriculture, University Putra Malaysia (UPM) during January to April, 2013 and all chemical analyses were carried out at the Food Biotechnology and Functional Food Research Laboratory, Faculty of Food Science and Technology, UPM, Malaysia.

2.2. Planting, cultural practices and sampling

Seedlings of the two common purslane and cuttings of the 10 ornamental purslane accessions (as ornamental purslanes do not produce seed) were first grown in plastic trays filled with rice field top soils (38.96% sand, 11.05% silt and 49.88% clay) with pH 4.8, 2.64% organic carbon, 1.25 g cc^{-1} bulk density and CEC of 7.06 me 100 g^{-1} soil. Soil nutrient status was 0.17% total N, 5.67 ppm available P, 15.6 ppm available K, 3357 ppm Ca and 319 ppm Mg. Soil water retention was 30.72% (wet basis) and 46.17% (dry basis) at field capacity. The soil belonged to the Serdang series.

Ten-day-old five seedlings or cuttings for each accession were transplanted in plastic pots (24 × 22 × 20 cm) filled with the same rice field top soil mentioned above. The plants were allowed to recover from transplanting shock and for full establishment for 30 days. During this time, plants were irrigated with tap water as and when necessary. No fertilizer was used. Five levels of salinity (0, 8.0, 16.0, 24.0 and 32.0 dS m^{-1}) were used in this study which were prepared using NaCl (Merck, Darmstadt, Germany) and distilled water. Salt treatment was initiated according to the treatments 30 days after transplanting (DAT) and continued till end of the study. In each pot, 200 mL of saline water was applied on alternate days according to the treatment. The control plants received 200 mL of distilled water. The experiment was organized in a two-factor (Pursale accessions × salinity) factorial randomized complete block design with three replications. Whole plants were harvested at the ground level at 60 DAT. The plants were washed under tap water, freeze-dried, ground and stored at −20 °C. Dry weights of the whole plants for each treatment and replication were recorded before grinding. The freeze-dried tissue was used for total phenolics, flavonoids and antioxidant capacity analyses.

2.3. Sample preparation and extraction

The extracts were prepared following methods described by Crozier, Lean, McDonald, and Black (1997) with slight modifications. Two grams of dry powdered purslane sample was weighted out in a 100 mL conical flask and 20 mL of methanol was added and left for 2 h in a water bath shaker with 100 rpm at 40 ± 1 °C temperature. The filtrate was separated from the residue by filtering through a filter paper (Whatman No. 1). The residue was re-extracted again with fresh solvent according to the procedure mentioned above. The filtrates were pooled and surplus methanol was then evaporated off under reduced pressure using a rotatory evaporator (Buchi Rotavapor R-210, Switzerland). The concentrated extract was then stored at −20 ± 1 °C for analysis.

2.4. Determination of antioxidant compounds

2.4.1. Determination of total phenolics compounds (TPC)

The TPC were determined using Folin–Ciocalteu method as reported by Singleton, Orthofer, and Lamuela-Raventós (1999) with slight modification. A 0.5 mL of sample extract was mixed with 0.5 mL Folin–Ciocalteu reagent, followed by addition of 10 mL of 7% Na_2CO_3 solution. The mixture was incubated for 1 h at 25 ± 2 °C in the dark and then absorbance was measured at 725 nm using a UV–Vis Spectrophotometer (UV-1650 PC Spectrophotometer, Shimadzu, Japan). The amount of TPC was expressed as milligram of Gallic acid equivalents (GAE) per g of dry weight of sample.