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NPK MEDIATED IMPROVEMENT IN BIOMASS PRODUCTION, PHOTOSYNTHESIS AND Na^+ REGULATION IN *PANICUM ANTIDOTALE* UNDER SALINE CONDITIONS

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Abstract

Panicum antidotale is a promising grass species for use as fodder and forage using non-conventional agricultural practices. It was therefore grown using brackish water irrigation to determine the optimal spacing and fertilizer treatment required for sustainable growth, ion relations and photosynthesis. Planting distance of 1.5 ft^2 resulted in higher biomass yield. Composite NPK fertilizer (NPK120) @ 120 kg ha^{-1} supported better plant growth rather than N, P, K individually or their various combinations (NP, NK, PK, NPK). Addition of fermented farmyard manure (NPK120+FM) improved ion regulation (lower Na^+ uptake, higher K^+/Na^+ ratio), photosynthetic rates and water use efficiency but did not improve biomass production compared to NPK120. However, higher Na^+ uptake with NPK120 would result in lower ion accumulation in the root zone and delayed soil degradation. We conclude that NPK120 could support sustainable growth of *Panicum antidotale* in our cropping system by keeping leaf Na^+ within safe limits for CO_2 assimilation and reducing the need for frequent re-planting of salinized root stock.

Key words: Cash crop; Fodder; Forage; Halophytes; High salinity

Introduction

Climatic changes and mismanagement of resources are resulting in increased soil salinity of prime agricultural lands with far reaching socio-economic consequences like food scarcity, sub-standard living and poor health, especially in arid and semi-arid regions (Qadir *et al*., 2008; Schade & Pimentel, 2010). In addition, water scarcity in arid climates could undermine efforts for sustainable development and threaten human health, ecosystems and national economies of the developing countries (Koyro *et al*., 2008). About 6% (more than 800 million hectares) of the world lands are affected by salinity, either due to salt accumulation over extended periods of time or to secondary salinity that has affected about 2% (32 million hectares) of the dry land-farmed areas and 20% (45 million hectares) of the irrigated lands in the world (Munns & Tester, 2008). Most of our field crops are salt sensitive while halophytic grasses are ideal fodder candidates on salt affected lands due to their salinity tolerance and natural ability to exclude Na^+ from shoots (Marcum & Murdoch, 1994). Differences in Na^+ uptake of grasses could vary with the nature and severity of salinity which affects availability of nutrients and consequently the plant yield. The use of nitrogen + phosphorus + potassium (NPK) fertilizers in different combinations enhances crop yield (Asghar *et al*., 2010; Law-Ogbomo & Law-Ogbomo, 2009) and has also been reported to improve yield of some halophytes in the presence of salinity (Noaman, 2004; Noaman & El-Haddad, 2000). Monitoring plant physiological traits such as photosynthesis and mineral uptake could be helpful in identifying optimal growth conditions for saline agriculture (Eshghizadeh *et al*., 2012).

Panicum antidotale Forssk (Poaceae), previously identified as *P. turgidum*, is distributed in dry and/or moderately saline environments (Cope, 1982), It has recently been recommended as an ideal non-conventional fodder grass crop for cultivation in saline fields with brackish water irrigation (Khan *et al*., 2009). At 125 mM NaCl biomass production of *P. antidotale* was unaffected due to improved gas exchange and water use efficiency when grown in a quick check system (Koyro *et al*., 2013). Information on the effects of additional nutrient treatments on growth, and physiological traits under field conditions could help in optimizing sustainable yield of this grass for commercial utilization. This study investigates the effects of various planting distances, straight and combined fertilizers of N, P and K on growth, ion regulation and gas exchange parameters of *Panicum antidotale* when grown in saline soil irrigated with brackish water.

Materials and Methods

Description of the study site and site preparation: All field trials were carried out at the Zia Model Farm, Hub Kund, 85 km north-east of the University of Karachi, Pakistan (24°58′17″ N; 66°46′33″ E), 240 m above sea level, ~10 km from the Balochistan coast. The climate here is semi-arid with less than 200 mm of annual rainfall. Generally, monsoon rains occur July through September with occasional winter rains (December to February). Average summer and winter day/night temperatures range between 35-25°C and 20-15°C, respectively. The soil is saline (EC 10-12 dS m^{-1}), silty loam with <1% organic matter. The field was cleared of the native vegetation, ploughed and leveled. *Panicum antidotale* root stocks collected from the adjoining area were used along with 0.5 ft seedlings of *Suaeda fruticosa* which were planted around each plot as a companion crop to absorb salts from the soil (as shown in Khan *et al*., 2009). The *Panicum antidotale* seedlings usually took 25-30 days to establish with a survival of about 60-70%. The salinity was too low to have any adverse effect on *Suaeda fruticosa* seedlings which were harvested periodically and removed from the site.

Irrigation water: Brackish underground water (EC 10-15 dS m^{-1}) was pumped up with the help of a wind mill and stored in a fiber glass tank for use when needed to irrigate the experimental plots. A diesel pump was also available for use in less windy days.

Fermenter: Towards one end of the field, a cemented sub-surface, open tank (5′x5′x5′) was constructed. Here, farmyard manure and poultry droppings (2:1) were fermented for 30 days in brackish water and irrigation water was passed through this pond before being channeled to the specific plots.

Spacing trial: *Panicum antidotale* was planted at distances of 1, 1.5 or 2 ft between plants and rows in 15 ft^2 plots, accommodating 225, 100 or 49 plants $plot^{-1}$ respectively. All plots received NPK fertilizer (Engro fertilizers) @ 120 kg ha^{-1} spread uniformly, 30 days after planting and irrigated fortnightly. The grass was harvested 30 days after the fertilizer application and their fresh weight recorded.

Effect of composite NPK fertilizer with or without fermented farmyard manure (FM): *Panicum antidotale* seedlings were allowed to establish for 30 days after transplanting. Various dosages of NPK fertilizer (40:40:40, Engro Fertilizers) @ 30, 60 or 120 Kg ha^{-1} were applied in pre-determined plots in a Randomized Complete Block Design with three replicates and irrigated fortnightly with underground brackish water either directly or after passing through the fermenter. The grass was harvested 30 days after the fertilizer application and their fresh weight recorded.

Treatment with individual (N, P, K) nutrients and their combinations (NP, KP, KN and NPK): Individual fertilizers (N, P and K) were applied individually as urea (46% N, Engro Fertilizers), single super phosphate (17% P, Sarhad Corporation) and potassium sulphate (50% K, Premium Fertilizers) respectively @ 120 kg ha^{-1}. Additionally, their various combinations (quantities in kg ha^{-1}) i.e., N+P (60+60), N+K (60+60), P+K (60+60) and N+P+K (40+40+40) were also applied in field plots in a Randomized Complete Block Design with three replicates. Plant biomass $plot^{-1}$ was determined 30 days after fertilizer application.

Photosynthesis: Net photosynthetic rates were measured using a LiCor 6400 portable photosynthesis system fitted with a 2x3 cm^2 open chamber. Photosynthetically active radiation of ~1000 µmol m^{-2} s^{-1} were maintained by orienting the sample chamber at an angle to the incident solar radiation. Flow rate of 500 µmol s^{-1} and VPD of ≤ 2.5 were maintained.

Shoot Na^+, K^+ and Ca^{++}: Hot-water extracts were prepared by mixing finely ground oven-dried plant material and homogenizing in deionized water at 100°C in capped Pyrex test tubes. Na^+, K^+ and Ca^{++} were determined on suitable dilutions of the hot water extracts by atomic absorption spectrometry (AA-700; Perkin Elmer, Santa Clara, CA, USA).

Statistical analyses: SPSS ver. 10 (SPSS Inc.) was used for analysis of variance (ANOVA) to test for variations among treatments and the post-hoc Bonferroni test ($p<0.05$) was used to determine significant differences between individual treatment means (Anon., 2001).

Results

Spacing: The 1.5 ft^2 spacing yielded biomass similar to 1 ft^2 which was ~2.2 fold higher than the 2 ft^2 spacing treatment (Fig. 1). The number of root stock for 1 ft^2 planting were almost twice that of 1.5 ft^2 treatment with no improvement in yield, hence the latter spacing was considered appropriate and less labor intensive.

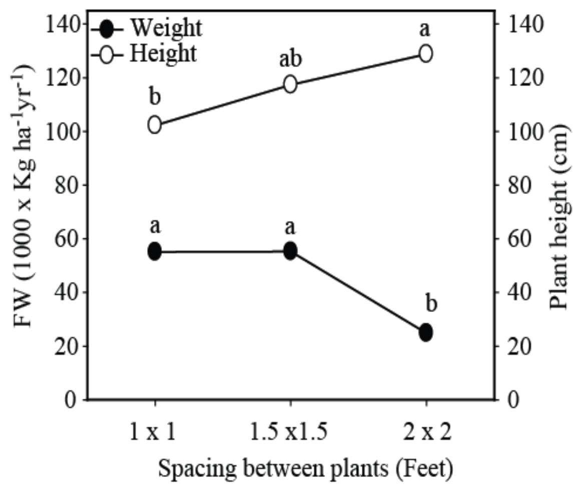

Fig. 1. Effect of spacing between plants on height and biomass (FW) of *P. antidotale*. Symbols with different letters represent significant differences at $p<0.05$ (Bonferroni test).

Effect of composite NPK fertilizer with or without FM: Among the various NPK dosage treatments used in this study, NPK120 alone or in combination with farmyard manure (FM) resulted in a 5 fold increase in biomass compared to control (Fig. 2). However plots with 60 kg ha^{-1} NPK + fermented farmyard manure (FM) tended to have a slight edge in terms of plant biomass yield compared to their counterparts where farmyard manure was missing (Fig. 2).

Effect of individual (N, P, K) nutrients and their combinations (NP, KP, KN and NPK): Combined treatment with N+P+K produced around 2 fold greater biomass than untreated controls. Among the other fertilizer treatments, nitrogenous fertilizer alone and in combination with K generally resulted in higher biomass yield (Fig. 3).

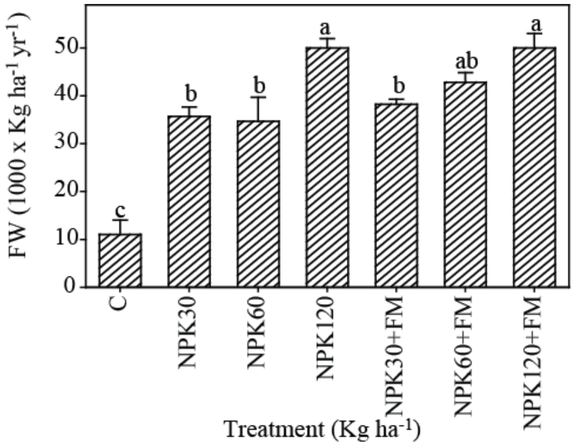

Fig. 2. Effect of increasing doses (30 to 120 Kg ha^{-1}) of NPK fertilizers with and without Fermented Farmyard Manure (FM) on fresh biomass (FW) of *P. antidotale* in comparison with untreated controls (C). Bars with different letters represent significant differences at p<0.05 (Bonferroni test

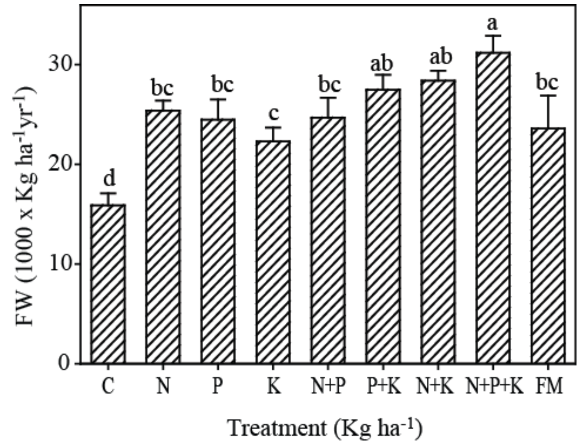

Fig. 3. Effect of individual (N: Nitrogen; P: Phosphorus; K: Potassium; FM: Fermented Farmyard Manure) and different combination of fertilizers on fresh biomass (FW) of *P. antidotale* in comparison with untreated controls (C). Bars with different letters represent significant differences at p<0.05 (Bonferroni test).

Table 1. Effect of increasing doses (30 to 120 Kg ha^{-1}) of NPK fertilizers with and without Fermented Farmyard Manure (FM) on net photosynthesis (Pn; µmol CO_2 m^{-2} s^{-1}), stomatal conductance (Gs; mol H_2O m^{-2} s^{-1}), inter-cellular CO_2 (Ci; µmol CO_2 m^{-2} s^{-1}), transpiration (E; mmol H_2O m^{-2} s^{-1}) and water use efficiency (WUE; µmol CO_2 $mmol^{-1}$ H_2O) of *P. antidotale*.

Treatments	Pn	Gs	Ci	E	WUE
Control	13.74 ± 1.5^{b}	0.15 ± 0.01^{bc}	203.83 ± 20.4^{a}	3.08 ± 0.2^{b}	91.6 ± 5.2^{d}
NPK30	18.83 ± 2.0^{ab}	0.21 ± 0.01^{a}	200.00 ± 09.4^{a}	4.28 ± 0.1^{a}	89.6 ± 3.6^{d}
NPK60	18.76 ± 2.1^{ab}	0.20 ± 0.01^{ab}	208.75 ± 21.5^{a}	4.46 ± 0.2^{a}	93.8 ± 5.4^{cd}
NPK120	22.62 ± 1.5^{a}	0.23 ± 0.01^{a}	190.67 ± 13.2^{ab}	4.78 ± 0.2^{a}	98.3 ± 4.3^{c}
NPK30 + FM	12.77 ± 3.4^{b}	0.12 ± 0.00^{c}	171.07 ± 48.1^{b}	2.44 ± 0.0^{c}	106.4 ±3.1^{c}
NPK60 + FM	18.83 ± 1.7^{ab}	0.13 ± 0.02^{c}	105.80 ± 32.5^{c}	2.52 ± 0.3^{c}	144.8 ±11.0^{b}
NPK120 + FM	26.23 ± 6.1^{a}	0.13 ± 0.04^{c}	160.40 ± 18.3^{b}	2.43 ± 0.6^{c}	201.8 ±7.2^{a}

Means ± standard errors followed by different letters are significantly different at p<0.05 (Bonferroni test)

Table 2. Effect of increasing doses (30 to 120 Kg ha^{-1}) of NPK fertilizers with and without Fermented Farmyard Manure (FM) on cations (Na^+, K^+ and Ca^{++}) in mg Kg^{-1} FW of *P. antidotale*.

Treatments	Na^+	K^+	K^+/Na^+	Ca^{++}
Control	156.3 ± 1.12^{ab}	165.2 ± 1.21^{a}	1.06^{c}	65.3 ± 1.21^{b}
NPK30	144.2 ± 1.34^{bc}	123.2 ± 3.21^{d}	0.85^{d}	68.2 ± 2.41^{b}
NPK60	133.2 ± 1.21^{cd}	144.2 ± 1.23^{c}	1.08^{c}	55.3 ± 1.68^{bc}
NPK120	166.9 ± 3.21^{a}	156.3 ± 1.23^{b}	0.94^{c}	42.3 ± 1.23^{cd}
NPK30 + FM	123.2 ± 2.21^{de}	122.3 ± 2.23^{d}	0.99^{c}	85.2 ± 1.21^{a}
NPK60 + FM	113.3 ± 4.21^{e}	160.4 ± 2.62^{b}	1.46^{a}	32.1 ± 2.31^{d}
NPK120 + FM	134.2 ± 2.21^{cd}	167.2 ± 1.23^{a}	1.23^{b}	65.2 ± 2.21^{b}

Means ± standard errors followed by different letters are significantly different at p<0.05 (Bonferroni test)

Photosynthesis: Photosynthetic rates were significantly (p<0.001) higher in NPK120 (22.6 µmol m^{-2} s^{-1}) and NPK120+FM (26.2 µmol m^{-2} s^{-1}) treatments (Table 1). Stomatal conductance and inter-cellular CO_2 concentrations were substantially (p<0.001) lower in NPK + FM indicating better stomatal regulation and carboxylation efficiency. Consequently, water use efficiency was generally higher in NPK + FM treated plants in comparison with control and NPK without FM (Table 1).

Shoot Na^+, K^+ and Ca^{++}: Plants accumulated lower Na^+ in leaves when treated with NPK+FM compared to all other treatments (Table 2). K^+ and K^+/Na^+ also decreased significantly (p<0.01) in NPK without FM in comparison with untreated control and NPK + FM treatments.

Discussion

A number of studies have addressed issues related to improvement in crop yield by better management and cultural practices. However, sustainability of a cropping system needs extended experiments on plant performance under ambient conditions. *Panicum antidotale* is an ideal fodder grass which grows optimally at moderate salinities (~100-150 mM NaCl) and produces up to 60,000 kg ha^{-1} yr^{-1} of biomass (Khan *et al*., 2009; Koyro *et al*., 2013). Salinity is known to reduce plant growth due to salt and/or drought related stresses by altering plant physiological functions. Growth of *Panicum antidotale* remained unaffected by $\leq$150 mM NaCl by enhancing photosynthesis, transpiration and water use efficiency and by using Na^+ as an osmoticum at both root and shoot tissues (Hussain *et al*., unpublished data). However, higher salinity reduced growth by decreasing chlorophyll content, rubisco and PSII activity through ROS production (Koyro *et al*., 2013). Salinity reduces soil fertility by limiting plant N uptake and lowering soil osmotic potentials (Irshad *et al*., 2002). Optimal use of NPK fertilizers enhanced crop yield and forage quality by improving the mineral balance in the absence of salinity (Asghar *et al*., 2010; Law-Ogbomo & Law-Ogbomo, 2009) and by minimizing Na^+ toxicity under saline conditions (Abusuwar & Al-Solimani, 2013). Initial trials with NPK showed the most promising effects on plant yield compared to individual (N, P, K) and combined (NP, PK, NK) effects of fertilizer treatments. Similar results were reported for other halophyte grasses when treated with nitrogen and potassium fertilizers (Noaman, 2004).

The application of manure has long been known to preserve and improve soil physical, chemical, and biological properties and also provide additional nutrients including N for plant (Irshad *et al*., 2002). However, little is known about the effects of NPK and FM on the growth and physiology of salt tolerant grasses. The highest yield with NPK120 treatment was similar to that with NPK120 + FM indicating that the optimal nutrient requirements were achieved by plants. Application of NPK alone resulted in enhanced photosynthesis coupled with higher stomatal conductance and transpiration. However, NPK120 + FM increased photosynthesis with higher water use efficiency. The relatively improved gas exchange parameters in NPK120 + FM treatment compared to NPK120 alone could be due to the higher cost of maintenance respiration.

Salt tolerance in grasses is generally associated with low uptake and accumulation of Na^+ in roots (Marcum, 2008). Lower Na^+ accumulation due to NPK120 + FM treatment compared to the NPK120 treatment indicates better ability for ion exclusion in the presence of additional N. Salinity generally reduces the uptake of essential ions (Ahmed *et al*., 2013), however, leaf K^+ was maintained in *P. antidotale* with the application of fertilizer treatments. Salt tolerant accessions of *P. antidotale* grown in 150 mM NaCl appeared to maintain relatively higher K^+/Na^+ ratios (Ahmad *et al*., 2010). A three folds increase in leaf Ca^{++} could be a possibility for K^+/Na^+ homeostasis (Sun *et al*., 2010).

Fermented animal manure mixed with irrigation water was used as readily available source of additional NH_4^+ and NO_3^- besides organic N which is the chief constituent of solid cattle manure (Tarkalson *et al*., 2006). Exact availability of nutrients is difficult to determine due to the influence of various factors like temperature, oxygen content and type of organic material on microbial decomposition and mineralization and need to be estimated under local conditions. Use of dissolved organic matter was preferred to avoid over or under application of N which could vary between 1 to 50 % for organic N (Power & Doran, 1984). However, additional sources of N may lead to nitrate toxicity for grazing animals (Ahmad *et al*., 2010).

Lower root Na^+ accumulation would make the cropping system more efficient due to less frequent need to replace aging root stock. Eco-physiological traits can be useful as biomarkers for efficient management of saline irrigation system in semi-arid environments. Appropriate amounts of NPK fertilizers need to be applied to obtain high yields of fodder crop. However, additional nutrient sources should only be used after weighing out the cost benefit ratio and sustainability of the cropping system.

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References

Abusuwar, A.O. and S.J. Al-Solimani. 2013. Effect of chemical fertilizers on yield and nutritive value of inter cropped *Sorghum bicolor* and *Lablab purpureus* forages grown under saline conditions. *J. Anim. Plant. Sci.,* 23: 271-276.

Ahmad, M.S.A., M. Ashraf and Q. Ali. 2010. Soil salinity as a selection pressure is a key determinant for the evolution of salt tolerance in Blue Panic grass (*Panicum antidotale* Retz.). *Flora*, 205: 37-45.

Ahmed, M.Z., T. Shimazaki, S. Gulzar, A. Kikuchi, B. Gul, M.A. Khan and K.N. Watanabe. 2013. The influence of genes regulating transmembrane transport of Na^+ on the salt resistance of *Aeluropus lagopoides*. *Funct. Plant Biol.*, 40: 860-871.

Anonymous. 2001. SPSS 10 for Windows Update. SPSS Inc., Chicago, USA

Asghar, A., A. Ali, W.H. Syed, M. Asif, T. Khaliq and A.A. Abid. 2010. Growth and yield of maize (*Zea mays* L.) cultivars affected by NPK application in different proportion. *Pak. J. Sci.,* 62: 211-215.

Cope, T.A. 1982. Poaceae, In: *Flora of Pakistan*. (Ed.): E. Nasir and S.I. Ali. Karachi University press, Pakistan.

Eshghizadeh, H.R., M. Kafi and A. Nezami. 2012. The mechanisms of salinity tolerance in the xero-halophyte Blue Panicgrass (*Panicum antidotale* Retz). *Not. Sci. Biol.*, 4: 59-64.

Irshad, M., S. Yamamoto, A.E. Eneji, T. Endo and T. Honna. 2002. Urea and manure effect on growth and mineral contents of maize under saline conditions. *J. Plant Nutr.*, 25:189-200.

Khan, M.A., R. Ansari, H. Ali, B. Gul and B.L. Nielsen. 2009. *Panicum turgidum*, a potentially sustainable cattle feed alternative to maize for saline areas. *Agri. Eco. Environ*., 129: 542-546.

Koyro, H.W., N. Geissler., S. Hussin and B. Huchzermeyer. 2008. Survival at extreme locations: life strategies of halophytes-the long way from system ecology, whole plant physiology, cell biochemistry and molecular aspects back to sustainable utilization at field sites, In: *Biosaline Agriculture and Salinity Tolerance in Plants*. (Ed.): A. Chedlly, M. Öztürk, M. Ashraf and C. Grignon. Verlag, Switzerland, Birkhaüser, pp. 241-246.

Koyro, H.W., T. Hussain, B. Huchzermeyer and M.A. Khan. 2013. Photosynthetic and growth responses of a perennial halophytic grass *Panicum turgidum* to increasing NaCl concentrations. *Environ. Exp. Bot.,* 49: 377-386.

Law-Ogbomo, K.E. and J.E. Law-Ogbomo. 2009. The performance of *Zea mays* as influenced by NPK fertilizer application. *Not. Sci. Biol.*, 1: 59-62.

Marcum, K.B. 2008. Relative salinity tolerance of turfgrass species and cultivars. In: *Handbook of turfgrass management and physiology*. (Ed.): F.L. Boca Raton, CRC Press, pp. 389-406.

Marcum, K.B. and C.L. Murdoch. 1994. Salinity tolerance mechanisms of six C4 turfgrasses. *J. Am. Soc. Hortic. Sci.,* 119: 779-784.

Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. *Ann. Rev. Plant Biol.*, 59: 651-681.

Noaman, N.M. 2004. Effect of nitrogen and potassium fertilizers on the growth and biomass of some halophytes grown under high levels of salinity. *J. Agron.*, 3: 25-30.

Noaman, M.N. and E.S. El-Haddad. 2000. Effects of irrigation water salinity and leaching fraction on the growth of six halophyte species. *J. Agri. Sci.,* 135: 279-285.

Power, J.F. and J.W. Doran. 1984. Nitrogen use in crop production. In: *Nitrogen in Crop Production*. (Ed.): H.D. Hauck and W.I. Madison, ASA, CSSA, SSSA, p. 589.

Qadir, M., A. Tubeileh, J. Akhtar, A. Larbi, P.S. Minhas and M.A. Khan. 2008. Productivity enhancement of salt-prone land and water resources through crop diversification. *Land Degr. Dev.*, 19: 429-453.

Schade, C. and D. Pimentel. 2010. Population crash: prospects for famine in the twenty-first century. *Environ. Dev. Sustain.*, 12: 245-262.

Sun, J., M.J. Wang, M.Q. Ding, S.R. Deng, M.Q. Liu C.F. Lu, X.Y. Zhou, X. Shen, X.J. Zheng, Z.K. Zhang, J. Song, Z.M. Hu, Y. Xu and S.L. Chen. 2010 H_2O_2 and cytosolic $Ca2^+$ signals triggered by the PM H^+-coupled transport system mediate K^+/Na^+ homeostasis in NaCl-stressed *Populus euphratica* cells. *Plant Cell Environ*., 33: 943-958.

Tarkalson, D.D., J.O. Payero, S.M. Ensley and C.A. Shapiro. 2006. Nitrate accumulation and movement under deficit irrigation in soil receiving cattle manure and commercial fertilizer. *Agr. Wat. Manage.,* 85: 201-210.

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