INTERNATİONAL JOURNAL OF AGRİCULTURE & BİOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
10–305/AWB/2011/13–1–38–42
http://www.fspublishers.org

Full Length Article

Effects of Microorganisms, Hormone Treatment and Stratification on Seed Germination of Goldenrain Tree (*Koelreuteria paniculata*)

MURAT ERTEKIN[1]
Department of Silviculture, Faculty of Forestry, University of Bartın, 74100, Bartın, Turkey
[1]Corresponding author's e-mail: muratertekin@hotmail.com

ABSTRACT

This study was conducted in an attempt to break dormancy and thus enhance germination of goldenrain tree (*Koelreuteria paniculata*) seeds. Prior to sowing, seeds were treated as follows: (i) cold stratification of seeds at 4°C for 30, 45 and 60 days; (ii) soaking in 500 mg/L polystimulin (PS-A6+PS-K), gibberellic acid (GA_3), or benzylaminopurine (BAP) for 24 h and stratification for 30 days; or (iii) soaking in 100 mL/L effective microorganisms (EM 1), 5000 mg/L PS-A6+PS-K, GA_3 and BAP for 72 h, followed by stratification for 45 days. Results indicated that the highest germination (94%) was obtained using 100 mL/L EM 1 application and stratification for 45 days at 4°C. Stratification was also effective for breaking dormancy of *K. paniculata* seeds with EM 1 and 45 days or 60 days of stratification. © 2011 Friends Science Publishers

Key Words: *Koelreuteria paniculata*; Germination; Pre-treatment; Ornamental plant

INTRODUCTION

Goldenrain tree (*Koelreuteria paniculata* Laxm.) is native to China, Korea and Japan. It is a small deciduous tree that is also called Pride-of-India, China tree, or varnish tree. The tree is deciduous and 9 m or taller, with feathery leaves 6–18 in (15–45 cm) long and racemes of yellow flowers (Lamb *et al*., 1985). Leaves change from yellowish to reddish in autumn. Yellow flowers occur from July to September in broad, loose, terminal panicles on trees in parks and gardens. The fruits are bladder-shaped, triangular, three-celled capsules. Fruit colour changes from green to light yellowish-red. Within the papery walls of ripe fruit are three round, black seeds (Rudolf, 1974; Pamay, 1992). It is a woody perennial, mainly planted in urban parks and gardens, and it has been cultivated since 1763, chiefly for ornamental purposes (Rehman & Park, 2000a).

Propagation of goldenrain tree from seed is difficult, because of strong dormancy. The dormancy can be due to seed coat, embryo, or a combination of both. Seed coat-imposed dormancy may be due to non-permeability to water and/or gases, mechanical prevention of radicle extension, or prevention of inhibitory substances from leaving or reaching the embryo. In embryo-imposed dormancy, there is usually a requirement for hormonal, temperature and/or light treatment that must be satisfied naturally during a period of after-ripening. The seeds of this species have both coat-imposed and embryo dormancy (Rudolf, 1974; Park & Rehman, 1999) and thus require treatments to overcome both forms of dormancy (Garner, 1979; Rehman & Park, 2000b; Bonner, 2008). Seed scientists and technologists have used various methods to break seed dormancy (Bewley & Black, 1994; Hartmann *et al.*, 1997) e.g., stratification, leaching, scarification, light and plant growth regulators (Bradbeer, 1988; Bonner *et al.*, 1994; Nowag, 1998). Several germination stimulators have also been applied to improve seed germination e.g., GA_3 (Dhankhar *et al.*, 1996; Vijaya *et al.*, 1996; Rahman *et al*., 2006; Soyler & Khawar, 2007), benzyladenine (Shafi *et al.*, 1991) and polystimulins (Kırdar & Ertekin, 2001).

Japanese experts have developed a mixture known as effective microorganisms (EM) to improve soils and plant growing conditions (Higa, 1991; Higa & Wididana, 1991). EM is a microbial inoculant comprised mainly of lactic acid and photosynthetic bacteria, yeasts and actinomycetes that are commonly found in soils (Higa, 1991). EM technology has been adopted globally and is recognised as a powerful and effective tool in both agriculture and horticulture for crop and animal production systems (Chamberlain *et al*., 1997). EM improves land use, irrigation water, seed yield and control of insect pests and diseases. The original product has had several name changes over the years e.g., "EM®", "EM-1®" and "Kyusei EM™".

Research on seed dormancy and germination in goldenrain tree is of great commercial and practical interest for nursery managers, because the results can be directly applied to improve techniques for propagating this species from seeds. Different pre-treatment have been used to

To cite this paper: Ertekın, M., 2011. Effects of Microorganisms, hormone treatment and stratification on seed germination of the goldenrain tree (*Koelreuteria paniculata*). *Int. J. Agric. Biol*., 13: 38–42

overcome goldenrain tree seed-coat non-permeability, such as mechanical scarification (Garner, 1979; Garner & Lewis, 1980), immersion in sulphuric acid (Rudolf, 1974), electromagnetic exposure (Maronek, 1975) and hormone treatment (Rehman & Park, 2000a, b). However, all pre-treatment methods applied to date do not totally overcome dormancy in this species. The present study examined the influence of several pre-treatments on germination of goldenrain tree seeds, with the goal of providing practical suggestions for breaking dormancy.

MATERIALS AND METHODS

Seed sources: Goldenrain tree capsules were collected from 10 trees planted in three seed lots, which were Ankara- Altın Park (39°57'N, 32°52'E, altitude 850 m above sea level), Bartın (41°37'N, 32°20'E, altitude 21 m above sea level) and Zonguldak-Ereğli (41°35'N, 32°19'E, altitude 15 m above sea level) in Turkey. Seed collection took place by late-September 2008 in the first year and by mid-October 2009 in the second year. Seeds were extracted by hand from capsules and cleaned with distilled water and seed fill was determined by floatation: filled seeds sink, while empty ones float. The filled seeds were used in all pre-treatment experiments.

First research year: In the first research year the role of the seed lots and the effects of stratification on germination were investigated. So, collected seeds from all seed lots were cold stratified (CS) in a beaker containing sand moistened with distilled water (DW) that were stored in a refrigerator at 4ºC for 30, 45 or 60 days.

Second research year: Study and analysis of the results of the first year demonstrated that there were also significant differences among the seed lots and seed-coat inhibited germination. Based on these conclusions, in the second year, collected seeds from Ankara-Altın Park seed lot were used. Seeds were CS in a beaker containing sand moistened with DW that was stored in a refrigerator at 4ºC for 30 or 60 days. Two hormone treatments were applied. In the first, seeds were imbibed in solutions (500 mg/L) of polystimulin (250 mg PS-A6 + 250 mg PS-K), gibberellic acid (GA_3) and benzylaminopurine (BAP) for 24 h at room temperature (20–25ºC) and stratified for 30 days at 4ºC. In the second experiment, seeds were imbibed in DW for 10 days at 40ºC then imbibed in solutions (5000 mg/L) of PS-A6 + PS-K (2500 mg each), GA_3 and BAP for 72 h at 25ºC and stratified for 45 days at 4ºC. The other pre-treatment was application of EM 1, in which seeds were imbibed in DW for 10 days at 40ºC then imbibed in a solution of 100 mL/L EM 1 for 72 h at 25ºC and stratified for 45 days at 4ºC.

The following treatments were used:

First research year

a) 40°C DW for 5 days + CS for 30 days in all seed lots
b) 40°C DW for 5 days + CS for 45 days in all seed lots
c) 40°C DW for 5 days + CS for 60 days in all seed lots.

Second research year

a) 40°C DW for 5 days + CS for 30 days
b) 40°C DW for 5 days + CS for 60 days
c) PS-A6 + PS-K (500 mg/L) for 24 h + CS for 30 days
d) GA_3 (500 mg/L) for 24 h + CS for 30 days
e) BAP (500 mg/L) for 24 h + CS for 30 days
f) 40°C DW for 10 days + 100 mL/L EM 1 for 72 h + CS for 45 days
g) 40°C DW for 10 days + PS-A6 + PS-K (5000 mg/L) for 72 h + CS for 45 days
h) 40°C DW for 10 days + GA_3 (5000 mg/L) for 72 h + CS for 45 days
i) 40°C DW for 10 days + BAP (5000 mg/L) for 72 h + CS for 45 days.

In the germination experiments, seeds of all treatments were placed in Petri dishes on two layers of filter paper moistened with 3% N-(trichloromethyl) thio-4-cyclohexene-1, 2-dicarboximide (Captan) to control fungal growth; these were placed in a growth chamber (MMM Clima Cell) with a daily photoperiod of 16 h light (175 μmol m^{-2} s^{-1}) at 24°C and 8 h dark at 18°C. Moisture was maintained with DW. Germination was monitored daily. Seeds with a radicle of at least 5 mm were considered germinated, removed from the Petri dish and counted.

All experiments were based on a completely randomized design, with three replications of 50 seeds each. Percentage data were subjected to arcsin transformation and one-way analysis of variance (ANOVA; SPSS ver. 9, SPSS Inc., USA) was performed (Zar, 1996). Differences among means were analysed with Duncan's multiple range test at $p = 0.01$.

RESULTS

Germination in the first research year: There was significant difference among seed lots in germination percentage (GP) ($P < 0.001$). Ankara-Altın Park seed lot showed a relatively greater germination with stratification than other seed lots (Bartın & Zonguldak-Ereğli). The highest GP was observed for the Ankara-Altın Park seed lot in pre-treatment of CS for 60 days at 4ºC (89%), followed by the Bartın (85%) and Zonguldak-Ereğli (81%) seed lots. Also, stratification duration is a crucial factor in seed germination. So, the best stratification duration was observed as 60 days in all seed lots (Fig. 1).

Germination in the second research year: The GP was significantly affected by all pre-treatments ($P<0.001$). The best GP (94%) was obtained from pre-treatment (f) (40°C DW for 10 days + 100 mL/L EM 1 for 72 h + CS for 45 days). Hence, EM 1 application was the most effective pre-treatment in the present study. Moreover, the GP differed significantly with stratification duration. Whereas 43% germination was obtained after 30 days stratification, while 60 days of stratification resulted in 90% germination (Figs. 2 & 3).

The other hormone treatments, apart from PS-A6 +

Fig. 1: Effects of seed lots and CS on germination of goldenrain tree seeds (*Numbers not followed by the same letter are significantly different at the 1% level, based on the Duncan criterion)

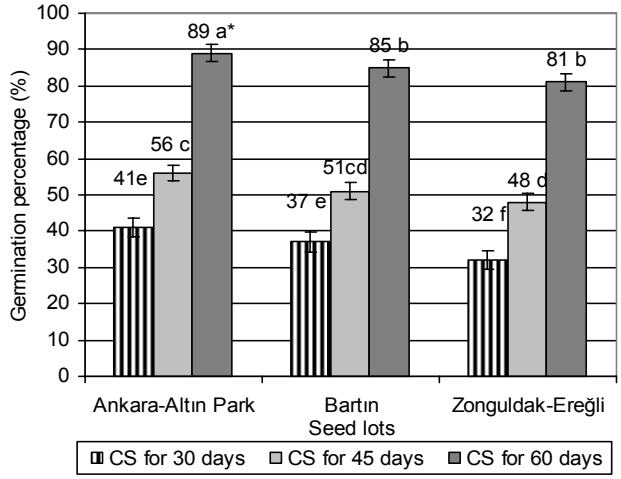
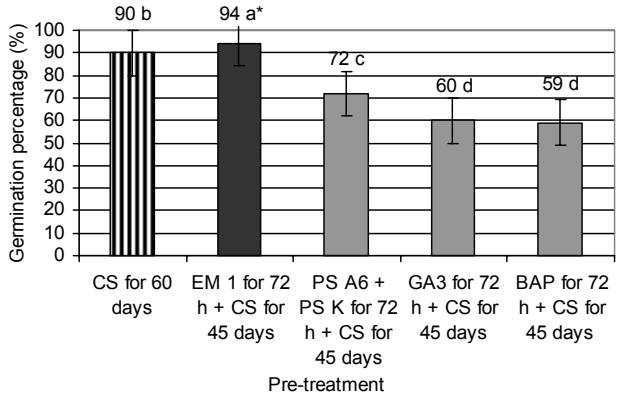


Fig. 2: Effects of pre-treatment on germination of goldenrain tree seeds



PS-K application, were not effective for germination. However, PS-A6 + PS-K application (47% GP) improved germination in comparison with cold stratification for 30 days (43% GP). GA_3 or BAP application had no significant effect on germination. Applications of 500 mg/L GA_3 or BAP also resulted in low GP (43% & 41%, respectively). Although PS-A6 + PS-K application was effective, it was insufficient to break dormancy of goldenrain tree seeds (Fig. 3).

DISCUSSION

As seen in this and previous studies, goldenrain tree seeds need pre-treatment before sowing (Rudolf, 1974; Rehman & Park, 2000a, b). In the present study, it was determined that seed lots and stratification durations affected seed germination percentage. Moreover, there was considerable germination variation among seed lots. For example, the highest mean germination (89% Ankara-Altın Park) for 60 days cold stratification treatment was significantly higher than the responses of all other seed lots. The difference in germination of seed lots may be related to environmental conditions associated with the seed lots such as altitude (Primac & Inouye, 1993; Hingston, 2000) or with higher seed fertility in Ankara-Altın Park than in Bartın and Zonguldak-Ereğli.

Fig. 3: Effects of hormone treatment and CS for 30 days on germination of goldenrain tree seeds

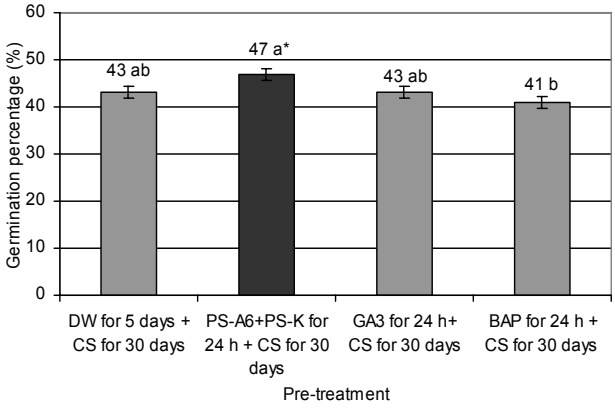


Fig. 4: Germination of goldenrain tree seeds

The best duration of the stratification varies among species and among different seed lots of the same species. There may be differences even within the same. Bonner (1991) informed that dormancy often appears to increase during storage and stored seeds require longer stratification than the same lots when fresh in southern pines. In the present study, the best germination was obtained from seeds stratified for 60 days (90%) and this varied significantly with stratification duration, with only 43% germination after 30 days of stratification. Hence, further pre-treatments were required for breaking dormancy. The applied pre-treatments were hormones or effective microorganisms (EM). The best GP (94%) was obtained from pre-treatment (f) (40°C DW for 10 days + 100 mL/L EM 1 for 72 h + CS for 45 days).

Therefore, EM 1 application was the most effective pre-treatment in the present study (Fig. 4).

The results show that application of EM 1 enhanced germination of the goldenrain tree to 94%. Studies on germination of this species are rare. Rudolf (1974) examined untreated seeds and found germination of only 2% after 29 days, whereas germination increased to 52% after acid plus stratification treatment. No unscarified seed germinated in any of the treatments, indicating that goldenrain tree seeds have hard, impermeable seed-coat dormancy.

Rehman and Park (2000a) reported that scarified seeds of goldenrain tree, without soaking or after soaking–redrying, had 44% germination after 60 days of moist chilling, which was increased to more than 50% when seeds were soaked or soaked–redried in DW or GA_3 for 5 h and moist chilled for 60 days (DW) and 30 days (GA_3). However, germination of seeds soaked for 24 h and moist chilled was very low, but increased if the seeds were redried after soaking. Dry chilling after soaking or soaking–redrying for 24 h also promoted germination and a maximum of >50% germination was achieved after 15 days of dry chilling. In other research, exogenous application of 100, 200 and 300 ppm GA_3 increased germination of scarified seeds from 0 (control) to 17, 18 and 15%, respectively. Pre-chilling in distilled water (DW) for 60 days increased germination to 44%. Compared with DW-chilled seeds, germination of seeds chilled for 15 days in GA_3 was significantly increased and germination of seeds chilled in 100, 200 and 300 ppm GA_3 was 60, 51 and 54%, respectively, after 30 days (Rehman & Park, 2000b). In the present study, high germination (94%) was obtained with the addition of EM 1. Seeds of cucumber, carrot, beet, tomato, pepper, corn, pea, burdock and bean were immersed for 10 min in undiluted EM and their germination was assessed. EM applied to tomato seeds gave 66% germination, while the control seeds had 6% germination; EM treatment significantly increased seed germination (Siqueira *et al.*, 1993). In addition, a positive effect of EM 1 on plant growth and development as well as on seed quality was observed (Konoplya & Higa, 1998).

Many investigations have reported that hormones are a controlling factor in seed dormancy and germination (Bewley & Black, 1994; Rascio *et al.*, 1998; Pascual *et al.*, 2009). Although in this study, the hormone treatments did not affect germination, PS-A6 + PS-K application (47% GP) improved germination in comparison with cold stratification for 30 days (43% GP). Besides, PS-A6 + PS-K is biologically active and meets the water and food needs of seeds by activating their metabolism. This treatment was also the most effective in *Magnolia grandiflora*, *Abies nordmanniana* and *Arbutus unedo* (Kırdar & Ertekin, 2001 & 2008).

In conclusion, 40°C DW for 10 days + 100 mL/L EM 1 for 72 h + CS for 45 days can successfully break dormancy in goldenrain tree seeds, resulting in the easy production of seedlings. Hence, goldenrain tree is a suitable choice for planting in urban parks and gardens.

REFERENCES

Bewley, J.D. and M. Black, 1994. *Seeds: Physiology of Development and Germination*, 2nd edition, p: 445. Plenum Press, New York

Bonner, F.T., 1991. Seed management. *In*: Duryea, M.L. and P.M. Dougherty (eds.), *Forest Regeneration Manual*, pp: 51–73. Kluwer Academic Publishers, Dordrecht, The Netherlands

Bonner, F.T., W.W. Vozzo and S.B. Land, 1994. *Tree Seed Technology Training Course*. USDA Forest Service GTR-SO-106, New Orleans, Louisiana

Bonner, F.T., 2008. Seed biology. *In*: Bonner, F.T. and R.P. Karrfalt (eds.), *Woody Plant Seed Manual*, pp: 3–38. USDA Forest Service Agriculture Handbook No. 727, Washington, DC

Bradbeer, W.J., 1988. *Seed Dormancy and Germination*. Blackie Acad., Glasgow, UK

Chamberlain, T.P., M.J. Daly and C.N. Merfield, 1997. Utilisation of Effective Microorganisms in Commercial Organic Agriculture: A Case Study from New Zealand. *Proc. 5th International Kyusei Nature Farming Conference*, pp: 120–123. Bangkok, Thailand

Dhankhar, D.S., K. Santosh and S. Kumar, 1996. Effect of bio-regulators on seed germination and seedling growth in aonla (*Phyllanthus emblica* Linn.) cv. Anand-2. *Recent Hortic.*, 3: 45–48

Garner, J.L., 1979. Overcoming double dormancy in golden-rain tree seeds. *Plant Propagator*, 25: 6–8

Garner, J.L. and A.J. Lewis, 1980. An evaluation of techniques used for germinating goldenrain tree seeds. *American Nurseryman*, 151: 12

Hartmann, H.T., D.E. Kester, F. Jr. Davies and R.L. Geneve, 1997. *Plant Propagation Principles and Practices*, 6th edition, p: 770. Prentice-Hall, Inc., Upper Saddle River, New Jersey

Higa, T., 1991. Effective microorganisms: A biotechnology for mankind. *In*: Parr, J.F., S.B. Hornick and C.E. Whitman (eds.), *Proc First International Conference on Kyusei Nature Farming*, pp: 8–14. U.S. Department of Agriculture, Washington, DC

Higa, T. and G.N. Wididana, 1991. The concept and theories of Effective Microorganisms. *In*: Parr, J.F., S.B. Hornick and C.E. Whitman (eds.), *Proc. First International Conference on Kyusei Nature Farming*, pp: 118–124. US Department of Agriculture, Washington, DC

Hingston, A.B., 2000. Are pollination syndromes useful predictors of floral visitors in Tasmania. *Australian Ecol.*, 25: 600–609

Kırdar, E. and M. Ertekin, 2001. The effects of PS-A6 and PS-K phytohormones and transplanting on seed germination and seedling growth of *Magnolia grandiflora* L. *Ener. Edu. Sci. Technol.*, 8: 17–23

Kırdar, E. and M. Ertekin, 2008. The role of polystimulin hormone application and stratification temperature to break the dormancy and improve seed germination for *Abies nordmanniana* (Stev.) Spach. *Seed Sci. Technol.*, 36: 301–310

Konoplya, E.F. and T. Higa, 1998. *Mechanisms of EM-1 Effect on the Growth and Development of Plants and its Application in Agricultural Production*. Institute of Radiobiology of the National Academy of Sciences of Belarus, University of Ryukyus, Japan

Lamb, J.G.D., J.C. Kelly and P. Bowbrick, 1985. *Nursery Stock Manual*, pp: 147–148. Growers Books, London

Maronek, D.M., 1975. Electromagnetic seed treatment increased germination of *Koelreuteria paniculata* Laxm. *Hortic. Sci.*, 10: 227–228

Nowag, A., 1998. Management of seed dormancy in *Fagus sylvatica, Fraxinus excelsior* and *Prunus avium*. *Comb. Proc. Int. Plant Propag. Soc.*, 48: 192–198

Pamay, B., 1992. *Bitki Materyali I*, p: 25. Ağaçlar ve Ağaççıklar Bölümü, Küçükkuyu, İstanbul, Turkey

Pascual, B., A. San Bautista, N. Pascual Seva, R. García Molina, S. López-Galarza and J.V. Maroto, 2009. Effects of soaking period and gibberellic acid addition on caper seed germination, *Seed Sci. Technol.*, 37: 33–41

Park, I.H. and S. Rehman., 1999. Studies on seed dormancy: Seeds maturation in relation to dormancy in goldenrain-tree (*Koelreuteria paniculata* Laxm.). *In:* Liptay, A., C.S. Vavrina and G.E. Welbaum (eds.), *Proc. Sixth Symposium on Stand Establishment and ISHS Seed Symposium*, pp: 199–208

Primac, R.B. and W.D. Inouye, 1993. Factors affecting pollinator visitation rates: a biogeographic comparision. *Curr. Sci.,* 65: 257–262

Rahman, M.H., M.S. Haque, M.A. Karim and M. Ahmed, 2006. Effects of gibberellic acid (GA_3) on breaking dormancy in Garlic (*Allium sativum* L.). *Int. J. Agric. Biol.,* 8: 63–65

Rascio, N., P. Mariani, F.D. Vecchia, N. La Rocca, P. Profumo and P. Gasraldo, 1998. Effects of seed chilling or GA_3 supply on dormancy breaking and plantlet growth in *Cercis siliquastrum* L. *Plant Growth Regul.,* 11: 201–209

Rehman, S. and I.H. Park, 2000a. Effect of Pre-Treatments on Dormancy of Goldenrain-Tree (Koelreuteria paniculata Laxm.) Seeds. *J. New Seeds*, 2: 29–36

Rehman, S. and I.H. Park. 2000b. Effect of scarification, GA and chilling on the germination of goldenrain-tree (*Koelreuteria paniculata* Laxm.) seeds. *Sci. Hortic.,* 85: 319–324

Rudolf, P.O., 1974. *Koelreuteria paniculata* Laxm., panicled goldenrain tree. *In*: Schopmeyer, C.S. and T. Coord (eds.), *Seeds of Woody Plants in the United States*, pp: 474–475. Agriculture Handbook, 450, USDA Forest Service, Washington DC

Shafi, B.M., A.Q. Shan and A.H. Lone, 1991. Propagation of *Magnolia grandiflora* L. through seed. *Prog. Hortic.,* 23: 30–33

Siqueira, M.F.B., C.P. Sudré, L.H. Almeida, A.P.R. Pegorer and F. Akiba, 1993. Influence of Effective Microorganisms on seed germination and plantlet vigor of selected crops. *In*: Parr, J.F., S.B. Hornick and M.E. Simpson (eds.), *In: Proc Third International Conference on Nature Farming, October 5-7*, pp: 222–245. Santa Barbara, California

Soyler, D. and K.M. Khawar, 2007. Seed germination of Caper (*Capparis ovate* var. *herbacea*) using α Naphthalene Acetic acid and Gibberillic acid. *Int. J. Agric. Biol.,* 9: 35–37

Vijaya, T., K.P. Srivasuki and P.S. Sastry, 1996. Role of gibberellic acid in teak seed germination and the effect of *Glomus macrocarpus* on growth and sodic soil tolerance. *Annl. For.,* 4: 211–212

Zar, J., 1996. *Biostatistical Analysis*, 3rd edition, pp: 277–284. Prentice-Hall, Inc., Upper Saddle River, New Jersey

(Received 22 June 2010; Accepted 17 July 2010)