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Salt Tolerance of *Rubia tinctorum* at Germination Stage

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Abstarct: A laboratory experiment was conducted to investigate the salinity tolerance of *Rubia tinctorum* at germination stage. The experimental design was randomized complete with seven levels of salinity and three replications. Salinity levels were 0, 3, 6, 9, 12, 15 and 18 dS/m. Forty sterilized healthy seeds of *R. tinctorum* were placed on a filter paper in a 9 cm sterilized Petri dish and 10 ml of prepared saline solution was added. They were placed in a germinator at dark with constant temperature of 25°C. For 22 days the germinated seeds were counted and the speed and percentage of germination were measured in each treatment. The results indicated that salinity reduced germination speed of *R. tinctorum*. The greatest delay in germination (9 days) was observed at 18 dS/m salinity treatment. Germination percentages up to 15 dS/m salinity level were not statistically different than that of control. The 50% germination was observed in 12 dS/m salinity treatment, which is suggested here as the salinity threshold value for germination stage of *R. tinctorum*. The results of this experiment suggest that *R. tinctorum* is salt tolerant during the germination stage.

Key words: Anthraquinones · Natural dye · Madder · Salinity

INTRODUCTION

Rubia tinctorum L., commonly known as madder, is a perennial herbaceous plant and a member of the Rubiaceae family [1]. The plant has been cultivated since ancient times for its value and importance as an industrial crop as well as its medicinal properties. The madder tops are used as forage crop harvested in the first and second year of cultivation [2]. The root of *R. tinctorum* is harvested in the third year which is highly valued for its high content of anthraquinone pigments [3]. The dye substance produced from the underground suckers of this plant includes pseudopurparin, rubiadin, minjistin, alizarin and purparin [4]. Anthraquinone pigments of madder roots have been used for dyeing textiles since 2000 B.C. [5]. The use of *R. tinctorum* root as herbal medicine has been reported [6, 7]. Extracts from *R. tinctorum* are used for the treatment of kidney and bladder stones [8, 9]. Madder roots, being rich in anthraquinones substance, have been widely used for pharmaceutical purposes such as treatment of kidney and bladder stones, as a laxative mixture and as a mild sedative [9-12]. It has reportedly also been used medicinally for menstrual and urinary disorders [12, 13]. Additionally, due to the heat and light resistant characteristics of anthraquinones [14], it has also been used in food processing industries [14-16].

R. tinctorum is indigenous to southern Europe, Middle East, Central Asia, Mediterranean region and North Africa [2, 12, 13]. Iran is known internationally as a main producer of madder [3]. It used to be cultivated in northwest, central part and south of the country. However, at present time it is mainly cultivated in some parts of Yazd province (central region of Iran), an arid and desert type area with scarce and saline water resources [2]. Approximately 90% of Iran, the second largest country in the Middle East, is classified as arid and semi-arid [17]. Shortage of fresh water resources, land salinization and water salinity are the major limiting factors for crop production in the country.

In recent years, increased attention has been paid to the use of saline soil and water resources for crop production. Cultivation of salt tolerant crops such as *R. tinctorum*, is a logical option for sustainable utilization of saline resources and conserving fresh water resources for conventional salt sensitive crops. Madder has been regarded as a salt tolerant crop by local farmers, however, scientific literatures on its salinity tolerance is quite

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scarce. Germination stage is important in determining the final number of plants per unit area and the necessary amount of seeds required for cultivation in the field. Therefore, the main objective of this study was to investigate the salinity tolerance of madder at germination stage. This could have practical implications for irrigation management of this crop with saline waters.

MATERIALS AND METHODS

A germination experiment was conducted in the laboratory at National Salinity Research Center (NSRC), Yazd, Iran. The experimental design was randomized complete with seven levels of salinity and three replications. Salinity levels were 0, 3, 6, 9, 12, 15 and 18 dS/m which were prepared by mixing water with 14 dS/m salinity from a well with deionized water (Table 1). The 15 and 18 dS/m salinity levels were prepared by first boiling the well water to increase its salinity and then mixing with deionized water to achieve the desired salinity levels.

Healthy seeds of *R. tinctorum* (local cultivar of Esmat) were sterilized and 40 of them were placed in a 9 cm sterilized Petri dish. A Watman filter paper was placed at the bottom of each Petri dish and after placing the seeds in them, 10 ml of prepared solution was added. After closing the Petri dishes, they were placed in a germinator at dark with constant temperature of 25°C. The dishes were inspected every other day and the filter papers and the stock solutions were replaced. For 22 days the germinated seeds were counted and the speed and percentage of germination were measured in each treatment. The results were statistically analyzed with SAS statistical package and the difference among treatments was analyzed by using Duncan's Multiple Range test at 5% probability level.

RESULTS AND DISCUSSION

The results indicated that salinity affects the germination speed of *R. tinctorum* (Fig. 1). Seeds in control (deionized water) started to germinate after 5 days, however, the time increased in other treatments. The greatest delay in germination (9 days) was observed at 18 dS/m saline water treatment (Fig. 1).

An interesting observation was that germination percentage of *R. tinctorum* increased at 3 and 6 dS/m in comparison to the control by 5 and 10.83% (Fig. 2). This was observed again when the experiment was repeated (data not shown). Although the increase was not statistically significant, but it could be investigated in future studies. It means that moderate levels of salt my

Table 1: Chemical analysis of the waters used in the experiment

		Anions (meq/l)				Cations (meq/l)				
Ec_i (dS/m)	pH	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	Na^+	K^+	SAR*
2	8.26	0.38	2.85	12.7	1.82	4.15	4.85	12.22	0.13	5.76
14	8.26	0.92	1.98	184.5	22.36	22.19	42.81	141.00	0.41	24.73

* Sodium Adsorption Ratio

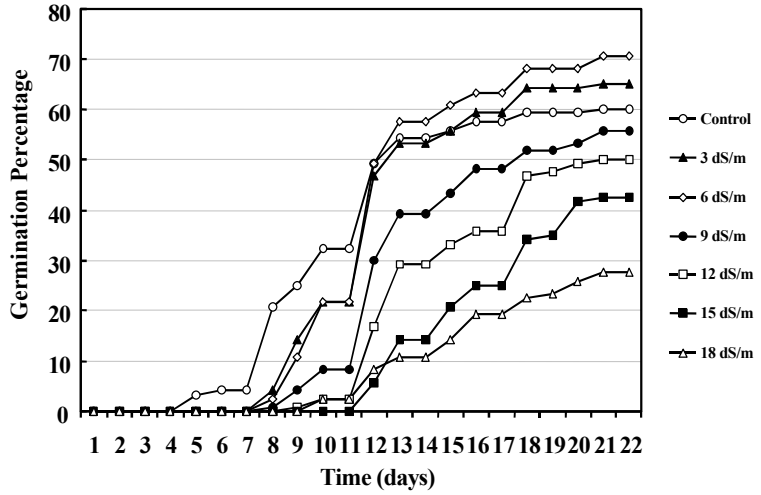

Fig. 1: The effect of salinity on germination speed of *Rubia tinctorum* seeds.

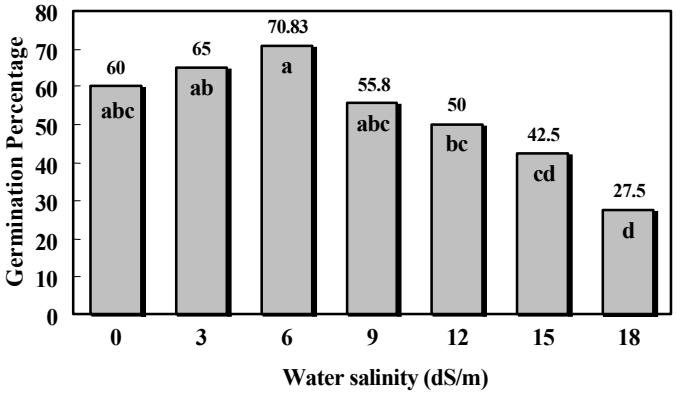

Fig. 2: Salinity effects on germination percentage of *Rubia tinctorum* seeds. Columns with the same letter are not statistically different than each other according to Duncan's Multiple Range test at 5% probability level

have enhancing effect on germination of madder, but higher levels have delaying and inhibitory effects on its germination percentage (Fig. 2).

The results showed that germination percentages up to 15 dS/m salinity level were not statistically different than that of control (Fig. 2). Abbasi *et al*. [18] reported that the threshold level at which nearly 50% of madder seeds did not germinate was 10 dS/m. Therefore, they suggested this value as the salinity threshold for germination of *R. tinctorum* [18]. In this experiment 50% germination was observed in 12 dS/m salinity treatment (Fig. 2), which is relatively close to the value reported by Abbasi *et al*. [18].

It should be mentioned that although germination percentage of 15 dS/m treatment was not significantly different than the control, but the seedlings looked quite fragile and weak. Therefore, we suggest 12 dS/m as the salinity threshold value for germination stage of *R. tinctorum*. The results of this experiment suggest that *R. tinctorum* is salt tolerant during the germination stage and saline waters of up to 12 dS/m maybe used for its germination.

REFERENCES

1. Gulhan, E.A. and K.M. Taskin, 1999. *Agrobacterium rhizogenes* mediated hairy root formation in some *Rubia tinctorum* L. population grown in Turkey. American J. Botany, 23: 373-377.
2. Sepaskhah, A.R. and Z. Beirouti, 2009. Effect of irrigation interval and water salinity on growth of madder (*Rubina tinctorum* L.). International J. Plant Production, 3(3): 1-16.
3. Baghalian, K., M. Maghsodi and M.R. Naghavi, 2010. Genetic diversity of Iranian madder (*Rubia tinctorum*) populations based on agro-morphological traits, phytochemical content and RAPD markers. Industrial Crops and Products, 31(3): 557-562.
4. Baslar, S. and H.H. Mert, 1999. Studies on the ecology of *Chrozophora tinctoria* L. and *Rubia tinctorum* L. in western Anatolia. Turkish J. Botany, 23: 33-44.
5. Angelini, L.G., L. Pistelli, P. Belloni, A. Bertoli and S. Panconesi, 1997. *Rubia tinctorum* a Source of Natural Dyes: Agronomic Evaluation, Quantitative Analysis of Alizarin and Industrial Assays. Industrial Crops and Products, 6(3-4): 303-311.
6. Hazara, B., M.D. Sarma and U. Sanyal, 2004. Separation method of quinonoid constituents of plants used in oriental traditional medicines. J. Chromatolography B., 812: 259-275.
7. Khalil, E.A., F.U. Afifi and M. Al-Hoseini, 2006. Evaluation of the wound healing effect of some Jordanian traditional medicinal plants formulated in pluronic F 127 using. J. Ethno. Pharmacol., 109: 104-112.
8. Westendorf, J., H. Marquardt, B. Poginsky, M. Dominiak, J. Schmidt and H. Marquardt, 1990. Genotoxicity of naturally occurring hydroxyanthraquinones. Mutation Res., 240: 1-12.
9. Blomeke, B., B. Poginology, C. Schmutte, H. Marquard and J. Westerndorf, 1992. Formation of genotoxic metabolites from anthraguinone glycosides, present in *Rubia tinctorum* L. Mutation Res., 265: 263-272.
10. Brown, J.P. and R.J. Brown, 1976. Mutagenesis by 9,10-anthraquinone derivatives and related compounds in *Salmonella typhimurium*. Mutation Res., 40: 203-224.

11. Mori, H., N. Yoshimi, H. Iwata, Y. Mori, A. Hara, T. Tanaka and K. Kawai, 1990. Carcinogenicity of naturally occurring 1-hydroxyanthraquinone in rats: Induction of large bowel, liver and stomach neoplasms. Carcinogenesis, 11: 799-802.
12. IARC (International Agency for Research on Cancer), 2002. *Rubia tinctorum*, *Morinda officinalis* and Anthraquinones. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Traditional Herbal Medicines, Some Mycotoxins, Naphthalene and Styrene, VOLUME 82, IARC Press, Lyon, France, pp: 129-151.
13. Medical Economics Co., 2000. PDR for Herbal Medicines, 2nd Ed., Montvale, NJ, pp: 80-81 and 490.
14. Ercan, A.G., K.M. Taskin, K. Turgut and S. Yuce, 1999. *Agrobacterium rhizogenes*-mediated hairy root formation in some *Rubia tinctorum* L. populations grown in Turkey. Turkish J. Botany, 23: 373-377.
15. Lodhi, A.H. and B.V. Charlwood, 1996. A. rhizogenes-mediated transformation of *R. peregrina* L.: In vitro accumulation of anthraquinones. Plant Cell, Tissue and Organ Culture, 46: 103-108.
16. Tereda, M., T. Terao and N. Koizumi, 2004. Evaluation Report Madder Color. Food Safety Commission, Food Additives Expert Committee, pp: 1-8.
17. Banakar, M.H. and G.H. Ranjbar. 2010. Evaluation of salt tolerance of pistachio cultivars at seedling stage. American-Eurasian J. Agric. And Environ. Sci., 9(2): 115-120.
18. Abbasi, F., A. Koochaki and A. Djafari, 2009. Evaluation of germination and vegetative growth of madder (*Rubia tinctorum* L.) under different levels of NaCl. Iranian J. Crop Res., 7(2): 515-525.