academicJournals

Vol. 8(6), pp. 254-259, June 2014
DOI: 10.5897/AJPS2013.1140
Article Number: AB8E2A145546
ISSN 1996-0824

http://www.academicjournals.org/AJPS

African Journal of Plant Science

Full Length Research Paper

Seed germination and abnormality of cotyledon of *Peganum harmala* populations in Mongolia

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Received 26 November, 2013; Accepted 3 June, 2014

The present study assessed whether seed germination of *Peganum harmala* L. is related to population size and whether factors that control three and four cotyledons and difference in cotyledon shape are different and whether abnormality of cotyledons is related to genetic drift. The percentage of final seed germination was highest in Dzungarian Gobian population and followed by Trans-Altai Gobian population and lowest was in East Gobian population (Tukey HSD test, p<0.01). Most seedlings of *P. harmala* have two cotyledons in our study areas but there were abnormal cotyledons which are three, four and different shape dicotyledons. Three and four cotyledons showed low frequency in biggest (limited by river basins) and bigger (limited by oasis) populations and high frequency in small population (limited by well) whereas pattern of different shape dicotyledon frequency does not follow difference of population size. The different shape dicotyledons showed higher frequency in region with lower elevation, as compared to regions with higher elevation. The present results suggest that percentage of seed germination of *P. harmala* and frequency of more than two cotyledons might depend on population size, while frequency of different shape dicotyledons might be related to habitat difference which is high soil salinity. Also, our results suppose more than two cotyledons might be related to genetic drift.

Key words: Abnormality of cotyledons, genetic drift, population size, seed germination.

INTRODUCTION

Seed germination is the last stage of embryogenesis and cotyledons are formed during embryogenesis of angiosperms (Goldberg et al., 1994; Gomez et al., 2006; Chandler, 2008). Previous studies described effects of temperature, water supply, light and salinity on seed germination of *Peganum harmala* L. These studies were done in the desert region, east of Cairo and the semi-desert Mediterranean coastal (Hammouda and Bakr, 1969) and Pakistan (Hussain and Nasrin, 1985). Menges (1991) reported that percentage of seed germination is related to population size.

The cotyledon functions primarily during seed germination and senescence shortly after the seedling emerges from the soil (Goldberg et al., 1994). Angiosperms have mono- or dicotyledons but more and less than two cotyledons have been reported in over 15

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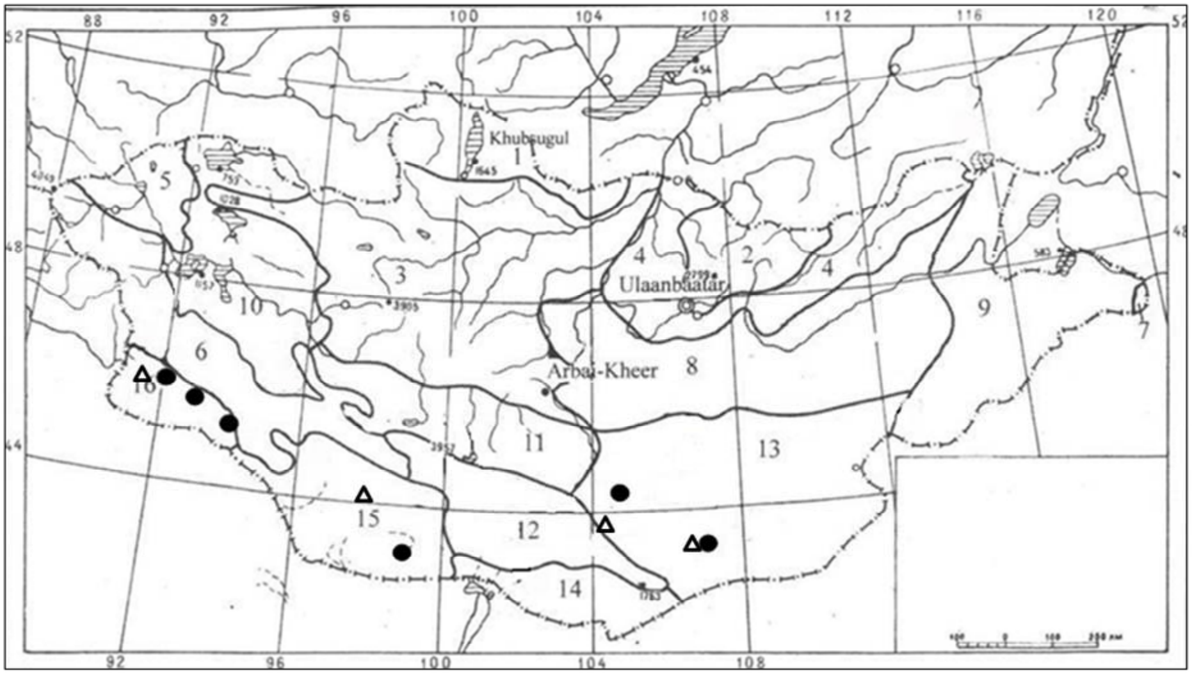

Figure 1. Seed samplng points of *P. harmala* in Mongolia (the botany-geographic region according to Grubov, 2001). ∆- weather station; seed sampling point. 1- Khubsugul, 2- Khentii, 3- Khangai, 4- Mongol-Dahuria, 5- Khovd, 6- Mongolian Altai, 7- Great Khingan, 8- Middle Khalkh, 9- Eastern Mongolia, 10- Great Lake Depression, 11- Valley of Lakes, 12- Gobi-Altai, 13-East Gobi, 14- Alasha Gobi, 15- Trans-Altai Gobi, 16- Dzungarian Gobi.

families (Conner and Agrawal, 2005).

The earliest defects were observed at the transition from the globular to the heart stage of embryogenesis with the formation of more than two cotyledons (Al-Hammadi et al., 2003). Previous studies supposed that cotyledon number range is related to hormonal asymmetric distribution and maternal effects (Liu et al., 1993; Mayer et al., 1993; Hadfi et al., 1998; Al-Hammadi et al., 2003; Orlova et al., 2006; Swarup et al., 2004), some kind of selection (Taylor and Mundell, 1999), lack of genetic variation (Conner and Agrawal, 2005), etc. Experimental studies reported that arabinogalactan proteins (AGPs) play functional roles in embryo development and cotyledon formation on *Nicotiana tabacum* (Qin and Zhao, 2007). Chandler (2008) reported that cotyledon bifurcation and lobing can result in supernumerary cotyledons. External nutrient (organic or mineral) supply during embryogenesis is important for embryo development (Qin and Zhao, 2007). However researchers still argue on the factor that could cause abnormality of cotyledon, most results supposed that genetic reasons might be related to abnormality of cotyledons.

Genetic variation was significantly reduced in endangered species when compared with non endangered species (Frankham, 1996). The effect of genetic drift is larger in small populations and smaller in large populations (Small et al., 2007). Dzungarian Gobi desert isolates distribution of *P. harmala* in Mongolia from a main distribution range in Middle Asia. In Mongolia, its distribution also isolated by deserts and then belongs to endangered species in Mongolia (Shiirevdamba et al., 1997).

The purpose of the present study was to assess whether seed germination of *P. harmala* is related to population size and whether factors that control more than two cotyledons and difference in cotyledon shape are different and whether abnormality of cotyledons is related to genetic drift.

MATERIALS AND METHODS

Seed samples were taken from three sites in the Dzungarian Gobi (46° 06’ 10.33N, 91° 35’14.49E, alt: 1191 m in Khovd river basin; 45° 53’ 03.28N, 91° 47’01.18E, alt: 1163 m in Unch river basin and 45° 34’ 34.89N, 93° 04’33.38E, alt: 1498 m near Shiir us spring) and one in Thras-Altai Gobi (43° 14’ 679N, 099° 00’ 411E, alt: 971 m in Ekhiin gol oasis) and two in East Gobi (44° 46’ 27.12N, 105° 15’32.55E, alt: 1200 m near well of herder camp between the sums Tsogt-Ovoo and Khongor and 43° 10’ 48.11N, 107° 10’29.36E, alt: 1060 m near well in Khanbogd sum, Southgobi aimag).

Climatic data is given based on database of Institute of Metereology and Hydrology of Mongolia. Nearest weather stations with seed sampling points were used for the present study: The nearest one with sampling points was “Baitag” station in Dzungarian Gobi and “Tooroi” station in Trans-Altai Gobi and “Dalanzadgad” and “Hanbogd” stations in East Gobi (Figure 1). Average temperature in July (°C) was 20.9, 23.4 and 21.2-23.8 and annual precipitation (mm) was 71.9, 49.5 and 84.0-127.1 in Dzungarian Gobi, Trans-Altai Gobi and East Gobi. Dzungarian Gobi

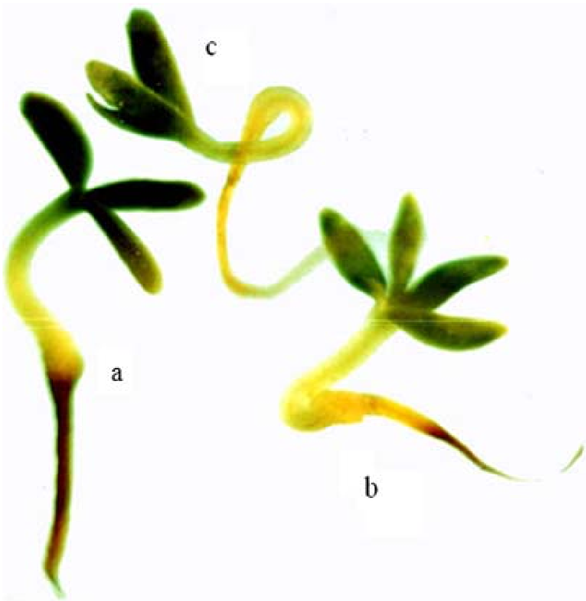

Figure 2. Diversity of *P. harmala* cotyledons in Mongolia. a- three cotyledons, b- four cotyledons, c- different shape dicotyledons.

region belongs to the province Dzungaria, Mts. Tien Shan and East Gobi and Trans-Altai Gobi belong to the province Mongolia, according to botany-geographic divisions (Grubov, 1963; Takhtajan, 1978).

The seed collection at the Institute of Botany was used for seed germination and observation of seedling morphology. In total, 6 seed samples were examined (Figure 1) which was collected between 1983 and 2001, from above-mentioned regions. When plants shed seeds, capsules were harvested then dried them paper bags at room temperature. The seeds were sampled randomly in each population.

Seed maturity of *P. harmala* was evaluated by weight of dry seeds as reported Harrington (1972). Weight of a thousand seeds was measured 10 times, using the analytic scale Shimadzu AY220 (d-0.1 mg). Seed germination was determined at 25±1°C for 10 days in the seed germinator, without dormancy breaking treatments, using Petri dishes and moist blotter by distilled water. Frequency of cotyledon number and shape was counted on 1000 embryos in each seed sample.

Maximum percentage of final seed germination was compared among regions, climatic variability and population sizes, because of only one site in Trans-Altai Gobi.

Population sizes (N) corresponded to the red-listed categories of “critically endangered” and “endangered”, “vulnerable” respectively (Brook et al., 2002). Main distribution range of *P. harmala* is in the Dzungaria, Mts. Tien Shan (Central Asia).

In Mongolia, population of this species in the Dzungaria is isolated from other populations by Gobi-Altai mountain system and Trans-Altai Gobian population is isolated from East Gobian population by Alasha Gobi desert. Distribution of *P. harmala* is limited to river basins, area of oasis and well in the Dzungarian Gobi, Trans-Altai Gobi and East Gobi regions. Hence, population size of *P. harmala* in river basins, oasis and near well was evaluated biggest (in the Dzungarian Gobi), bigger (in Trans-Altai Gobi) and small (in East Gobi), respectively.

Geographic differences of weight of a thousand seeds, final germination, frequency of different shape dicotyledons, three and four cotyledons were estimated by Tukey HSD test.

RESULTS

Mean of a thousand seed weight of *P. harmala* was 2.67, 2.43 and 2.6 g in the regions Dzungarian Gobi, Trans-Altai Gobi and East Gobi. Percentage of final seed germination was 95, 72 and 61% in the Dzungarian Gobi, Trans-Altai Gobi and East Gobi, respectively. To compare percentage of final seed germination with climatic variability, high and low percentage of seed germination was found in region of low and high air temperature in July but regional difference of its percentage does not follow regional difference of annual precipitation. The percentage of final seed germination was highest in Dzungarian population and followed by Trans-Altai Gobian population and lowest in East Gobian population (Tukey HSD test, $p<0.01$).

Most seedlings of *P. harmala* have two cotyledons in our study areas but there were abnormal cotyledons which are three (Figure 2a), four cotyledons (Figure 2b) and different shape dicotyledons (Figure 2c).

In comparison, frequency of abnormal seedlings among populations, 1.6, 2.7 and 6.8% of all seedlings in Dzungarian, Trans-Altai Gobian and East Gobian populations have three cotyledons but 0.5 and 1.5% in Dzungarian and East Gobian populations have four cotyledons. Different shape dicotyledons were found (9.6, 14 and 7.4%) in Dzungarian, Trans-Altai Gobian and East Gobian populations. Frequency of three cotyledons in East Gobian population was significantly higher than in other populations. However, frequency of three cotyledons was not significantly different between Dzungarian and Trans-Altai Gobian populations, Dzungarian population showed low frequency, when compared with Trans-Altai Gobian population. Also, frequency of four cotyledons was not significantly different between Dzungarian and East Gobian populations but its frequency was low in Dzungarian population, when compared with East Gobian one. Frequency of different shape dicotyledons was significantly higher in Trans-Altai Gobian population than in Dzungarian and East Gobian populations. However, frequency of different shape dicotyledons was not significantly different between Dzungarian and East Gobian populations, Dzungarian population showed higher frequency, when compared with East Gobian populations (Figure 3).

Different shape dicotyledons showed low and high frequency in region with high and low altitude but frequency of three and four cotyledons does not follow. To compare frequency of abnormality of cotyledons with climatic variability, high frequency of three and four cotyledons was found in regions with 21.2-23.8°C of average temperature in July while low frequency was found in regions with 20.9-23.4°C. Different shape dicotyledons showed high frequency in region with 49.5 mm of annual precipitation and low frequency in regions with 71.9-127.1 mm whereas frequency of three and four

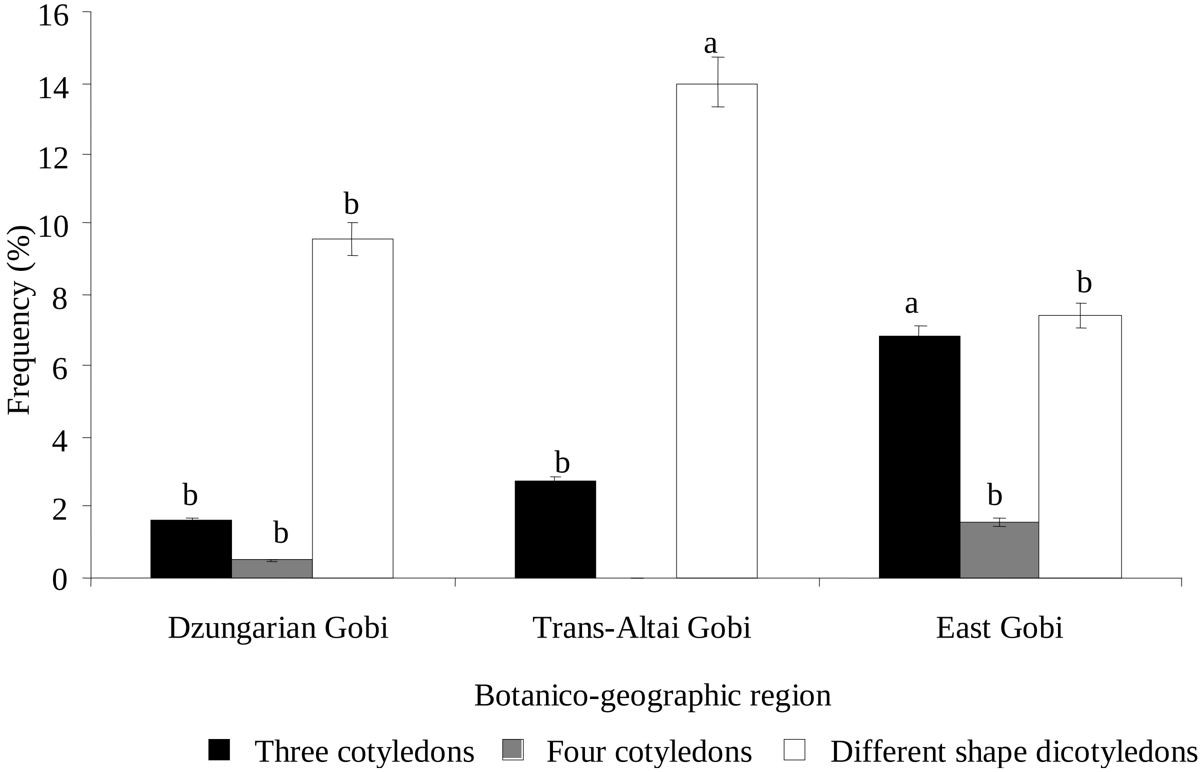

Figure 3. Cotyledon number and shape differences of *P. harmala* among the botany-geographic regions in Mongolia (Tukey HSD test, $p<0.05$).

cotyledons does not follow regional difference of annual precipitation.

Highest frequency of three and four cotyledons was found in East Gobian population with lowest final seed germination but that was lowest in Dzungarian population with highest final seed germination. The highest frequency of different shape dicotyledons was found in Trans-Altai Gobian population with averaged final seed germination whereas it was significantly low in Dzungarian and East Gobian populations with highest and lowest percentage of final seed germination. These patterns show that frequency of three and four cotyledons increased with decreasing percentage of final seed germination but frequency of different shape dicotyledons was not correlated with percentage of final seed germination.

Three and four cotyledons showed low frequency in biggest (limited by river basins) and bigger (limited by oasis) populations and high frequency in small population (limited by well) whereas pattern of different shape dicotyledon frequency does not follow difference of population size.

DISCUSSION

Seed germination is the most important factor for *P. harmala* growth and distribution. *P. harmala* seeds germinated well at about 10 mm of rainfall (Hammouda and Bakr, 1969). Daily amount of precipitation in our study sites is insufficient for seed germination of this species, resulting in low amount of annual precipitation. But, river, oasis and well water supply could be sufficient for seed germination.

P. harmala exhibits 40 and 80-90% of final seed germination at 25 and 30°C in room temperature (Hussain and Nasrin, 1985). The present experiment indicated that seeds of *P. harmala* germinated 60-95% at 25°C. To compare results of previous and present experiments, seed germination of *P. harmala* is higher in Mongolia than in Cairo, Mediterranean coastal and Pakistan at same temperature. It seems seeds of *P. harmala* in Mongolia could adapt to coldness, better than above mentioned regions. However, negative correspondence between percentage of final seed germination and experimental temperature was found, seeds of *P. harmala* showed highest percentage of germination in Dzungarian Gobi with lowest temperature in July, when compared with other regions. Hence, regional difference of annual precipitation and air temperature in July cannot be main limiting factors for seed germination of this species.

However, ground water supply is sufficient for seed germination of *P. harmala*, water supplying area must be different among river, oasis and well. The results indicate that seed germination of *P. harmala* is low and high in small and bigger populations, as reported by Menges (1991). Hence seeds of *P. harmala* cannot potentially germinate in Mongolian desert, because of area limitation.

Previous studies reported more and less than two cotyledons in the family Aceraceae, Juglandaceae, Rubiaceae, Pedaliaceae, Protaceae, Ranunculaceae, Papaveraceae, Brassicaceae, Fabaceae, Geraniaceae, Chenopodiaceae, Onagraceae, Solanaceae, Scrophulariaceae, Salicaceae and Euphorbiaceae (Gates, 1910; Went, 1944; Harrison, 1964; Dessureaux, 1967; Magsar and Tsagaanmaam, 1984; Rajora and Zsuffa, 1986; Taylor and Mundell, 1999; Graz, 2001; Conner and Agrawal, 2005; Chandler, 2008). The present results show three, four cotyledons and different shape dicotyledons in *Peganum* genus (Peganaceae), in addition to above mentioned families.

Experimental study on tomato showed that abnormality of cotyledons is formed during embryogenesis (Al-Hammadi et al., 2003). Qin and Zhao (2007) showed that βGlcY affects cotyledon formation of *Nicotiana tabacum* L. Recent studies explain abnormality of cotyledons as a lack of genetic variation (Conner and Agrawal, 2005) but researchers still argue on factors and genetic reasons of abnormality of cotyledons (Chandler, 2008).

Frequency of more than two cotyledons showed same pattern with regional difference of average temperature in July but frequency of different shape dicotyledons showed negative pattern with regional difference of annual precipitation and elevation. The correspondences suppose that frequencies of more than two cotyledons and different shape dicotyledons might be signs of different factors, showing frequency of more than two cotyledons corresponding to heating that cause water evaporation while frequency of different shape dicotyledons was with length of vegetation period that depends on elevation.

Frequency of more than two cotyledons negatively corresponded with percentage of final seed germination. Seed maturation was not different among regions, because of seed weight indifference (Tukey HSD test, $p>0.05$). Correspondence between frequency of more than two cotyledons and population size indicated that frequency of more than two cotyledons decreases towards the main distribution range of *P. harmala*. This results suppose that more than two cotyledon might be related to genetic drift. Several genes such as DRN/ESR1 and DRN-LIKE/ESR2 have a role in cotyledon development, with mutant showing syncotyly and pleiocotyly (Chandler, 2008).

Cytokinin and arabinogalactin proteins (AGPs) play an important role during the stage of embryogenesis at which cotyledon number is determined (Chaudhury et al., 1993; Qin and Zhao, 2007). The present study discuss canalization of cotyledon number and bifurcation or lobbing as cause of more than two cotyledons formation (Conner and Agrawal, 2005; Chandler, 2008).

More than two cotyledons means shape of cotyledon leaves on a seedling of *P. harmala* is similar while different shape dicotyledons means shape of two cotyledon leaves on a seedling is different. The different shape dicotyledons showed higher frequency in region with lower elevation (in oasis), as compared to in regions with higher elevation. High value of average temperature in July and low value of annual precipitation indicated drought condition in Trans-Altai Gobi. Hence, *P. harmala* in oasis used more ground water for growth during drought season. However, soil salinity in oasis is higher than other dry regions, resulting in ground water evaporation (Pankova et al., 2004; Pankova, 2008). Safina (1977) reported that calyx lobe of *P. harmala* was found in high saline habitats (in oasis) but it was complete in low saline habitats and showed it as polymorphism.

Water stress induced by drought affects β-amylase activity of cucumber cotyledons in non-saline soil condition (Todaka et al., 2000) but our results suggest that different shape dicotyledons in *P. harmala* might depends on soil salinity which explains osmotic and toxicity effects, in saline soil condition.

The present results suggest that percentage of seed germination of *P. harmala* and frequency of more than two cotyledons might depend on population size, while frequency of different shape dicotyledons might be related to habitat difference which is high soil salinity. Also, our results suppose more than two cotyledons might be related to genetic drift.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The author thanks Professor G. Tserenbaljid and academician Ch. Dugarjav (Institue of Botany) for advice and comment on this study and the writing of the manuscript.

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