

Cotyledon Numbers of Two *Peganum* Species (Peganaceae) in Mongolia

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Abstract

Most seedlings of *Peganum harmala* and *P. nigellastrum* have two cotyledons, but up to 10% of all seedlings of *P. harmala* have three cotyledons, and up to 1.7% has four cotyledons. Up to 5.4% on *P. nigellastrum* have only three cotyledons. Frequency of three cotyledonous seedlings among these two species was significantly different. The present results report three cotyledonous seedlings, as well as four cotyledonous seedlings in the family Peganaceae, in addition to two cotyledonous seedlings. On both species, the frequency of three cotyledonous seedlings was increased with increased annual precipitation and decreased air temperature, and in localities, which isolated from main distribution range. The frequency of three cotyledonous seedlings of *P. harmala* increased with increasing soil alkalization, but that of *P. nigellastrum* increased with decreasing alkalization.

Key words: *Peganum harmala*, *Peganum nigellastrum*, cotyledon, seedlings

Introduction

Most angiosperms are mono- or dicotyledonous, but three cotyledons have also been reported for plants of over 15 families (Conner & Agrawal, 2005). The number of cotyledons was unclear for species of Peganaceae van Tieghem.

Previous studies supposed that cotyledon number range is related with hormonal asymmetric distribution (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 & Mundell, 1999), lack of genetic variation (Conner & Agrawal, 2005), and maternal effects (Al-Hammadi *et al.*, 2003).

The purpose of this study is to seek whether three cotyledons occur in the family Peganaceae and to determine whether cotyledon number range is related with loss of habitat.

Materials and Methods

Climatic data is given according to the report of Namkhaijantsan (2009). Mean air temperature in July was fluctuated between 20 and 25°C in the Dzungaria, Valley of Lakes, Gobi Altai and north of East Gobi regions; between 10 and 15°C in the Mongol-Dahuria region, and more than 25°C in the Trans-Altai Gobi and East Gobi regions. Annual precipitation was 100-150 mm in Dzungaria, East

Gobi and Valley of Lakes; 150-200 mm in Gobi Altai; 50-100 mm in west of East Gobi region; less than 50 mm in Trans-Altai Gobi, and 300-400 mm in Mongol-Dahuria.

Soil pH is one of main factors for plant metabolism (Fink, 1976), and it was 7.4-8.0 (slight alkalic) in Valley of Lakes, Gobi Altai and east of East Gobi; 8.1-9.0 (strong alkalic) in Dzungaria, Trans-Altai Gobi, west of East Gobi and Mongol-Dahuria (Batkhishig, 2009).

The seed collection at the Institute of Botany, Mongolian Academy of Sciences was used for seed germination and seedling morphology. In total, 13 seed samples were examined (Table 1), collected between 1983-2007, from above-mentioned regions. Mongol-Dahuria and Dzungaria belong to the province Western Siberia and Dzungaria of Mts. Tien Shan, respectively, and Valley of Lakes, East Gobi, Gobi Altai, Trans-Altai Gobi belong to the province Mongolia, according to botany-geographic divisions (Grubov, 1963; Takhtajan, 1978).

When plants shed seeds, we harvested capsules then dried them in paper bags. The seeds were sampled randomly in the population.

Weight of thousand seeds was measured with 10 repetitions using an 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

Table 1. Information related with seed collecting sites

Botany-geographical regions	Site	Date	Latitude	Longitude	Altitude, m
<i>P. harmala</i>					
Dzungaria	near Shiir-Us spring	05 Sept. 1992	N45°34'34.89	E93°04'33.38	1498
Trans-Altai Gobi	Ekhiin gol oasis	28 Aug. 2001	N43°14'679	E099°00'411	971
East Gobi	between Tsogt-Ovoo and Khankhongor villages	2 Sept. 1987	N44°46'27.12	E105°15'32.55	1200
	near Khanbogd mountain	10 Sept. 1995	N43°10'48.11	E107°10'29.36	1060
<i>P. nigellastrum</i>					
Mongol-Dahuria	Altanbulag village	24 Sept. 2007	N50°31'840	E106°48'947	690
Valley of Lakes	Near Orog lake	19 Aug. 1998	N45°04'01.54	E100°55'05.16	1227
Gobi Altai	Near Bayanlig village	09 Aug. 1998	N44°34'47.68	E100°34'46.73	1479
	Khatan Suudal valley	08 Aug. 1998	N44°50'58.05	E102°23'17.05	1478
	Among the sand dunes	14 Aug. 2000	N44°04'37.31	E103°48'00.65	1304
	Moltsog Els				
	Bayanzag	14 Aug. 2000	N43°47'20.37	E104°09'55.29	1403
	Among the sand dunes	11 Aug. 2000	N43°46'047	E102°148'927	1359
	Khongoriin Els				
	Nogoon Khoshuu valley	05 Aug. 2000	N43°06'631	E102°19'869	1624
East Gobi	Near Ongon village	05 Sept. 1999	N45°21'14.08	E113°08'20.03	1066

with distilled water. Frequency of three and four cotyledons has counted on 1000 seedlings in each seed sample.

Main distribution range of *P. harmala* is in the Dzungaria, Mts. Tien Shan (Central Asia). This species is very rarely distributed in Mongolia. The main distribution range of *P. nigellastrum* is in Mongolia, but this is rarely distributed in Siberia.

Frequency of cotyledon number between species were compared by Mann-Whitney U-test (Avery, 2004), and between cotyledon number

range and precipitation, air temperature, soil pH by effect test, using statistic software JMP 4.0.

Results

Most seedlings of *P. harmala* and *P. nigellastrum* being with two cotyledons, but 10% of all seedlings of *P. harmala* bear up to three cotyledons, and 1.7% has four cotyledons (Fig. 1A). Up to 5.4% on *P. nigellastrum* have only three cotyledons (Fig. 1B). Frequency of three



Figure 1. Seedlings of *P. harmala* (A), *P. nigellastrum* (B). a-two cotyledonous seedling, b-three cotyledonous seedling, c-four cotyledonous seedling.

cotyledonous seedlings between two species was significantly different ($U=33$, $Z=2.31$, $P=0.02$).

Frequency of three cotyledonous seedlings in *P. harmala* was varied between 2.7-3.4% when annual precipitation was less than 100 mm, and mean of air temperature in July was more than 25°C, but that was 4.7-10.2% when annual precipitation is more than 100 mm and mean of air temperature is less than 25°C. This frequency in *P. nigellastrum* was 0.9-1.4% when

annual precipitation is less than 200 mm and mean of air temperature in July is more than 20°C while, the above value was 5.4% when annual precipitation was more than 200 mm and mean of air temperature was less than 20°C.

The frequency of three cotyledonous seedlings of *P. harmala* was more than 3.4% when soil pH is more than 8.1, whereas that was less than 3.4% when soil pH is less than 8.1. This frequency of *P. nigellastrum* has opposite relation (Fig. 2).

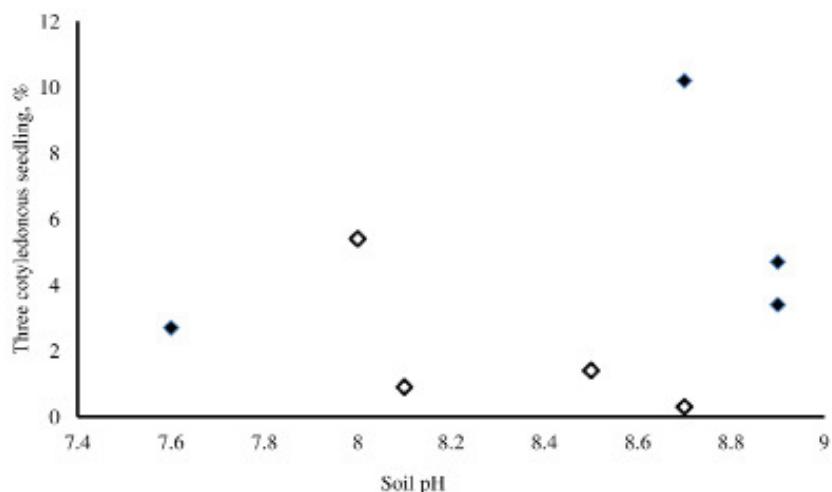


Figure 2. Correspondence between frequency of three cotyledonous seedlings and soil pH (Effect test, $DF=1$; $SS=252$; $F=7.1$; $P<0.0097$); solid square - *P. harmala*, open square - *P. nigellastrum*.

Three cotyledonous seedlings on *P. harmala* were occurred for 3.4-10.2% of all seedlings in East Gobi and Dzungaria, and 2.7% in Trans-Altai Gobi. The three cotyledonous seedlings in

P. nigellastrum were 0.3-1.4% in East Gobi, Gobi Altai and Valley of Lakes, but 5.4% in Mongol-Dahurian region (Fig 3a, b).

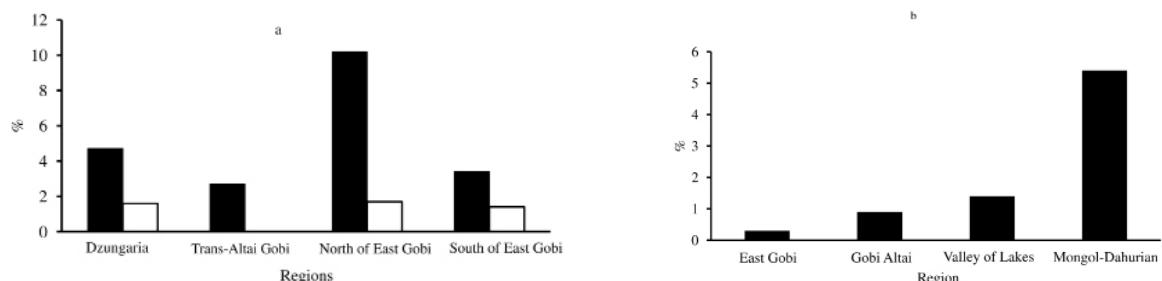


Figure 3. Frequencies of three and four cotyledonous seedlings on *P. harmala* (a) and *P. nigellastrum* (b) by the botany-geographical regions; solid bars – three cotyledonous seedlings, open bars – four cotyledonous seedlings.

Discussion

The three cotyledonous seedlings have been found before this study in many families such as 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 & Tsagaanmaam, 1984; Rajora & Zsuffa, 1986; Taylor & Mundell, 1999; Graz, 2001; Conner & Agrawal, 2005; Chandler, 2008). The present findings report not only three cotyledonous, but also four cotyledonous seedlings in the family

Peganaceae, in addition to two cotyledonous seedlings. The other authors reported that 5-69% and 19% of all seedlings might have three and four cotyledons, respectively based on selection experiments (Taylor & Mundell, 1999; Conner & Agrawal, 2005). Naturally, 0.5-5% of all seedlings bear more than two cotyledons (Magsar & Tsagaanmaam, 1984; Conner & Agrawal, 2005). Our results indicate that three and four cotyledonous seedlings can be found in more than 0.5% of all seedlings.

Cotyledon number range of *P. harmala* that is very rare in Mongolia was higher than that of common *P. nigellastrum* ($U=34$, $Z=2.45$, $P=0.01$), and the cotyledon number range of *P. harmala* was larger than that of *P. nigellastrum*. This might indicate that *P. nigellastrum* better adapted in Mongolia than *P. harmala*. Both species can be considered as adapted well in dry condition because of frequency of three cotyledonous seedlings on both species increased with increasing annual precipitation (effect test, $DF=1$; $SS=178$; $F=9.9$; $P<0.0344$) and decreasing air temperature (effect test, $DF=1$; $SS=198$; $F=11$; $P<0.0293$). The frequency of three cotyledonous seedlings of *P. harmala* increased with raise of soil alkalization, but that of *P. nigellastrum* increased with decreased alkalization. This means that *P. harmala* adapted in slight alkalic soil, but the other species is in strong alkalic soil. On the other hand, embryogenesis of *P. harmala* altered by increasing soil alkalization, whereas that of other species altered by decreasing alkalization.

Frequency of three cotyledonous seedlings of *P. harmala* was higher in the regions Dzungaria and Trans-Altai Gobi than in the East Gobi, while that of *P. nigellastrum* was higher in the Mongol-Dahurian region than other regions. Because of localities that are high frequency of three cotyledonous seedlings for both species isolated from main distribution range.

Al-Hammadi *et al.* (2003) reported that the earliest defects were observed at the transition from the globular to the heart stage of embryogenesis with the formation of multiple cotyledons. In this case, cotyledon number of *P. harmala* and *P. nigellastrum* can be related with embryogenesis. Embryogenesis of *P. harmala* altered in the wet and strong alkali condition while that of *P. nigellastrum* in the wet and slight alkali condition. Occurrence of three and four cotyledons show that embryogenesis of *P. harmala* altered stronger

than of *P. nigellastrum*. The embryogenesis of *P. harmala* in the region East Gobi altered stronger than in other regions, but that of *P. nigellastrum* in the Mongol-Dahurian region was stronger than in other regions. The results indicate that the cotyledon number range of *P. harmala* and *P. nigellastrum* is related with habitat loss, which is soil moisture gradient and soil alkalization.

References

- Al-Hammadi, A. S. A., Sreelakshmi, Y., Negi, S., Siddiqi, I. & Sharma, R. 2003. The polycotyledon mutant of tomato show enhanced polar auxin transport. *Plant Physiology*, 133: 113-125.
- Avery, L. 2004. Mann-Whitney U-test. <http://elegans.swmed.edu/~leon/stats/utest.cgi>. (Leon@eatworms.swmed.edu).
- Batkhisig, O. 2009. Map of soil pH. In: *National Atlas of Mongolia*, 2nd edition, Ulaanbaatar, p. 123. (in Mongolian)
- Chandler, J. W. 2008. Cotyledon organogenesis. *J. Exp. Bot.*, 59(11): 2917-2931.
- Conner, J. K. & Agrawal, A. A. 2005. Mechanisms of constraints: the contributions of selections and genetic variance to the maintenance of cotyledon number in wild Radish. *J. Evol. Biol.*, 18: 238-242.
- Dessureaux, L. 1967. Selection for pleiotropism in alfalfa. *Can. J. Genet. Cytol.*, 9: 658.
- Fink, A. 1976. Pflanzenemehrung in Stichworten. Kiel, Hirt, p. 80.
- Gates, R. R. 1910. Abnormalities in Oenothera. *Missouri Botanical Garden Ann. Rep.*, 21: 175-187.
- Graz, F. P. 2001. Three cotyledons on *Schinziophyton rautanenii* seedlings. *S. Afr. J. Bot.*, 67: 69-70.
- Grubov, V. I. 1963. *The plants of Central Asia*. Academy Press, Moscow-Leningrad, Russia.
- Hadfi, K., Steph, V. & Neuhaus, G. 1998. Auxin-induced developmental pattern in *Brassica juncea* embryos. *Development*, 125(5): 879-887.
- Harrison, B. J. 1964. Factors affecting the frequency of tricotyly in *Antirrhinum majus*. *Nature*, 201: 424.
- Liu, C., Xu, Z. & Chua, N. H. 1993. Auxin polar transport is essential for the establishment of bilateral symmetry during early plant embryogenesis. *Plant Cell*, 5(6): 621-630.

- Magsar, D. & Tsagaanmaam, D. 1984. Three cotyledon of *Adonis mongolica* Sim. *Proceedings of the Institute of Botany, Mongolian Academy of Sciences*, 10: 57-62. (in Mongolian)
- Mayer, U., Buttner, G. & Jurgens, G. 1993. Apical-basal pattern formation in the *Arabidopsis* embryo: studies on the role of the *gnom* gene. *Development*, 117(1): 149-162.
- Namkhajantsan, G. 2009. Maps of air temperature and precipitation. In *National Atlas of Mongolia*, 2nd edition, Ulaanbaatar, pp. 98, 105. (in Mongolian)
- Orlova, I., Marshall-Colyn, A., Schnepp, J., Wood, B., Varbanova, M., Fridman, E., Blackeslee, J. J., Peer, W. A., Murphy, A. S., Rhodes, D., Pichersky, E. & Dudareva, N. 2006. Reduction of benzoid synthesis in *Petunia* flowers reveals multiple pathways to benzoid acid and enhancement in auxin transport. *Plant Cell*, 18(2): 3458-3475.
- Rajora, O. P. & Zsuffa, L. 1986. A typical seedlings of *Populus* L: their genetic significance and value in breeding. *Silvae Genet.*, 35: 122-124.
- Swarup, R., Kargul, J., Marchant, A., Zadik, D., Rahman, A., Mills, R., Yemm, A., May, S., Williams, L., Millner, P., Tsurumi, S., Moore, I., Kerr, I. D. & Bennett, M. J. 2004. Structure function analysis of the presumptive *Arabidopsis* auxin permease *AUX1*. *Plant Cell*, 16(11): 3069-3083.
- Takhtajan, A. L. 1978. *The floristic regions of the world*. Nauka Press, Leningrad. (in Russian)
- Taylor, N. L. & Mundel, N. E. 1999. Registration of multiple-cotyledon red clover genetic marker stock: L38-1485. *Crop Science*, 39: 1259.
- Went, R. R. 1944. Morphological observations on the tomato plant. *Bull. Torrey Bot. Club.*, 71: 77-92.

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