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SOME SEED AND SEEDLING CHARACTERISTICS (TRICOTYLEDONY) OF *OPUNTIA FICUS INDICA* (L.) MILL. (CACTACEAE)

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ABSTRACT

The seeds of *Opuntia ficus indica* (L.) Mill. are rounded in outline with convex surfaces. The seed coat is hard and shiny. The seed weight follows normal distribution i.e., few seeds had very low weight (< = 14 mg), few had larger weight (> or = 22.1mg) and most of the seeds had weight ranging from 14.1 to 19.9 mg. Hardness and imperviousness of testa to water imposes dormancy. Germination of seeds is slow and very low but enhanced on H_2SO_4 scarification. Majority of seedlings had two cotyledons but few of them (11 in 140 seedlings i.e., 7.86%) exhibited tricotyledony. In dicotyledonous seedlings major spurt in expansion of both large and small cotyledons of the seedlings took place from 4 to 6 days after emergence – later the expansion curve became more or less asymptotic. In case of tricotyledonous seedlings expansion of the large, medium and small cotyledons appeared to be somewhat slow but continuous as observed during initial 15 days of growth. Tricotyledonous seedlings didn't differ significantly from dicotyledonous ones in radicle and hypocotyl growth but had somewhat larger cotyledonary area per seedling.

Key-words: *Opuntia ficus–indica*; seed weight distribution, germination and seedling growth, tricotyledony.

INTRODUCTION

Opuntia ficus-indica (L.) Mill., Prickly pear or Anarphali or Nagphani, is widely distributed cactus in the world (Nobel, 2002) including arid areas of Pakistan (Mirza and Bokhari, 1996; Saeed, 1999). This multipurpose species is tolerant to intense drought and heat. It grows on poor soils and is reported to be potentially useful in combating desertification (Mulas and Mulas, 2004). Potter *et al.* (1984) have investigated germination of some *Opuntia* spp. (*O. edwardsii, O. lintheimeri and O. discarta*). Some seed and seedling characteristics of the local provenance of *O. ficus-indica* from Northern Areas of Pakistan *are* investigated here to develop baseline ecological understanding of the in hand provenance of this species.

MATERIALS AND METHODS

Seeds of *O. ficus-indica* were collected from its fruits, which were in turn collected from Northern areas of Pakistan in the month of January 2006. The seeds were thoroughly washed with running water, air-dried and stored for around a month before experimentation.

Seed-Weight Distribution

Hundred randomly selected seeds were weighed individually to constant weight. The frequency distribution was characterized with location and dispersion parameters (Fisher, 1948).

Germination studies

Three Hundred randomly chosen seeds of *O. ficus-indica*, were sown in an earthen trough of 30 cm diameter containing 2 kg of well-fertilized garden sandy loam spread uniformly and placed in open for illumination. It was irrigated with tap water daily to keep the soil fairly moist. Prior to their sowing the seeds were scarified with conc. H_2SO_4 for 5 min. because several species of *Opuntia* are reported to bear hard-coated seeds whose germination is enhanced by chemical scarification (Potter e*t al*. 1984). A similar germination trial was run with unscarified seeds. Emergence counts were made daily for one month. Seedling characteristics, their growth and phenotypic variation amongst them were studied. In view of paucity of tricotyledonous seedlings, their cotyledonary area was determined by dimension measurement and employing relationship - Area of cotyledon = Length x breadth x 0.666 (Cain and Castro, 1959). In case of dicotyledonous seedlings, they were harvested at the specified time and cotyledonary outlines were drawn on graph paper for determination of cotyledonary area. The estimates by the two methods didn't vary by a quantum more than 5%.

RESULTS AND DISCUSSION

The seeds of *Opuntia ficus-indica* are round in shape with both surfaces convex. The testa is rough and shiny. As indicated by the chi-square analysis seed weight distribution is normal with low variability (CV= 18.2%). The weight of seeds concentrates around the mean value of 17.90 ± 0.33 mg (Fig. 1). In 84% of the cases seed weight ranged from 14.1 to 22 mg. These results are in close agreement with APAT (2003) who reported that number of seeds per Kg in *O. ficus-indica* to be ranging from 49000 to 55500 seeds, which corresponds to seed weight falling between 18.01 to 20.4 mg per seed. The homogenous distribution of *Opuntia* seed weight is in contrast to Harper *et al* (1970) but in agreement with Labouriau and Pacheco (1979) who found a lot of *Dolichos lablab* L. relatively uniform and dispersed-normally. The variability in seed weight in *Achyranthes aspera* and *Peristrophe bicalyculata* (Khan *et al*. 1984) and *Cassia holosericea* (Khan and Shaukat, 1990) has also been reported to be low. The implication of low seed weight variation in *O. ficus-indica may*, however, be adjudged as a significant factor in the population dynamics of this species because keen intraspecific competition among seedlings arising from seeds of closely similar weights.

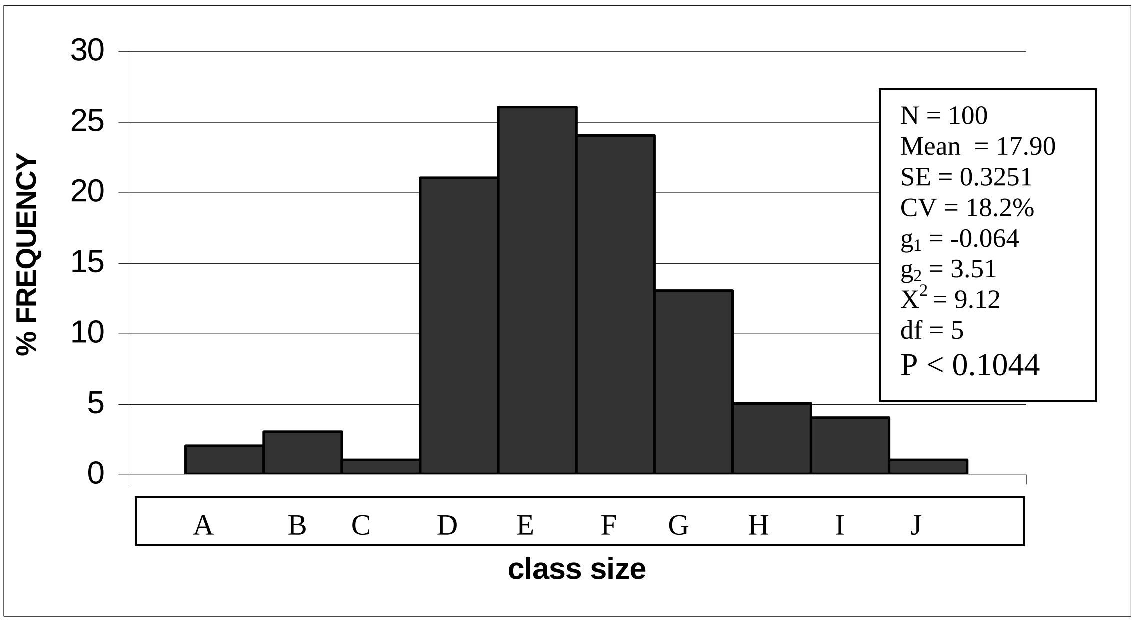

Fig. 1. Seed weight (mg / seed) distribution of *Opuntia ficus-indica*. Class sizes: A, 8 –10 mg; B, 10.1 –12 mg; C, 12.1 – 14, mg …….J, 26.1 to 28 mg per seed. The hypothesis that population is normal of mean 17.90 and SD 3.2513 cannot be rejected at the 95% confidence level.

It is apparent from germination studies (Fig. 2) that rate as well as final germination percentages of unscarified seeds remained significantly and remarkably low as compared to H_2SO_4 scarified seeds. The germination of seeds is gradual and lasts for several days. Acid scarification enhanced germination from 8% to c.39%. Presumably, hard and impervious seed coat of seeds prevented moisture availability to the embryo. H_2SO_4-scarification of seeds is reported to enhance seed germination in many opuntias- e.g., 15% in *Opuntia stricta* (Monteiro *et al.*, 2005) and 2.3 to 17- fold in *O. edwarsii, O. linthemeiri*, and *O. disca*rta (Potter *et al*. 1984). Improvement in germination by sulphuric acid in Carrbbean apple cactus (*Harrisia fragrans*) has also been reported (Dehgan, 2005). In *O. ficus-indica* the dormancy of seeds appears not to be imposed due to hard seed coat alone but also due to false aril that originates from funiculus and surrounds the ovule. It lignifies when seed is mature (Gallo and Quagliotti, 1989). The occurrence of different kinds of epicuticular waxes in form of plates and granules in micropylar region (Degano *et al.*, 1997) could also be a significant factor in germination ecology of this species. The removal of waxes by acid may presumably be permitting seeds to germinate due to moisture availability to the embryo.

The seedlings of *O. ficus-indica* are phenotypically non-uniform. Seedlings are slow growing with long juvenile period and start developing phylloclade only when they are around 40 - 45 days of age. Long juvenile period of seedlings is known to be common in *Opuntias* (Pimienta-Barrios,1990). Almost all of the seedlings had unequal cotyledons (Fig.3 &4), which are lanceolate in shape and acute to obtuse at the apex. The hypocotyl remains bent like the hood of snake at the time of emergence. Interestingly, at this age the larger cotyledon is always the upper cotyledon. On subsequent day of emergence, the hypocotyl becomes erect and cotyledons start separating. Polyembryony, as large as 4 seedlings per seed of *Opuntia*, as reported in literature (APAT, 2003), couldn’t be seen in our studies. In stead, out of 140 seedlings around 7.86% of the seedlings (11 in number *in toto*) exhibited tricotyly or tricotyledony (Fig. 3 & 4) - a rare phenomenon but so far reported in some 15 families of dicotyledonous plants

(Conner and Agrawal, 2005). Brancheley and Warrington (1936) reported tricotyledony in *Aphanes arvensis* L (1 tricotyl in 2700 seedlings; 0.037%), *Veronica hederifolia* L. (1 tricotyl in 1000 seedlings; 0.10%), *veronica arvensis* L. (1 tricotyl in 20,000 seedlings; 0.005%) and *Capsella bursa-pastoris* (1 tricotyl in 5,500 seedlings; 0.0182%). Tricotyledonous seedlings are known to sporadically occur in nurseries of lab lab (Ayangar and Nambiar, 1941), tomato (Reynard, 1952; Kerr, 1985), mustards (Holtorp, 1944), snapdragon (Harrison, 1964), etc. Dicotyledonous species such as *Decalepis hemiltonii* Wight & Arn. (www.nisc.air.res.in/science communication/Abstracting journals/isa/isa2k5/isa_1dec.05.asp),*Tectona grandis L. (http://forest.ap.nic.in/JFM%20CFM /implementation %20status*/Semi%20Annual%202005.htm), pepper (forums.gardenweb .com/forums /load /pepper /msgo31222381 4006.html?6), *Prosopis cineraria* (Nagesh *et al*., 2001a), *Acacia catechu* (www.trophort.com /information/ index074.html), *Acacia millifera* (Nagesh *et al.*, 2001b), *Sapindus emarginatus* (Nagesh *et al*., 2004) and *Populus balsamifera* L. (www.na.fs.fed.us/spfo/pub/silvics_manual/volume-2/populusbalsamifera.htm) are also reported to rarely bear three cotyledons. *Angophora floribunda* (Sm.) Sweet rarely produces tricotyledonous seedlings (Prakash, 1969). In wild radish (*Raphanus raphanistrum*), Conner and Agrawal (2005) reported the occurrence of pleiocotyly in 10 seedlings bearing greater than two cotyledons out of 1857 seedlings (0.54%). Recently, Hu *et al.* (2005) have reported spontaneous tricotyledonous mutant in *Helianthus annuus*. In view of comparatively higher percentage of Pleiocotyly (7.86%), cotyledon number in *O. ficus-indica* appears to be a less canalized trait.

The tricotyledonous mutants are reported to be controlled by single recessive gene in *Catharanthus roseus* (Rai and Kumar (2001) and due to a few mutated genes in *Arabidopsis thaliana* (Azumi *et.al.* 2002; Vernon *et.al.* 2001; Conway and Poethig, 1997). The tricotyledony in *O. ficus-indica* needs to be studied on genetic and molecular basis.

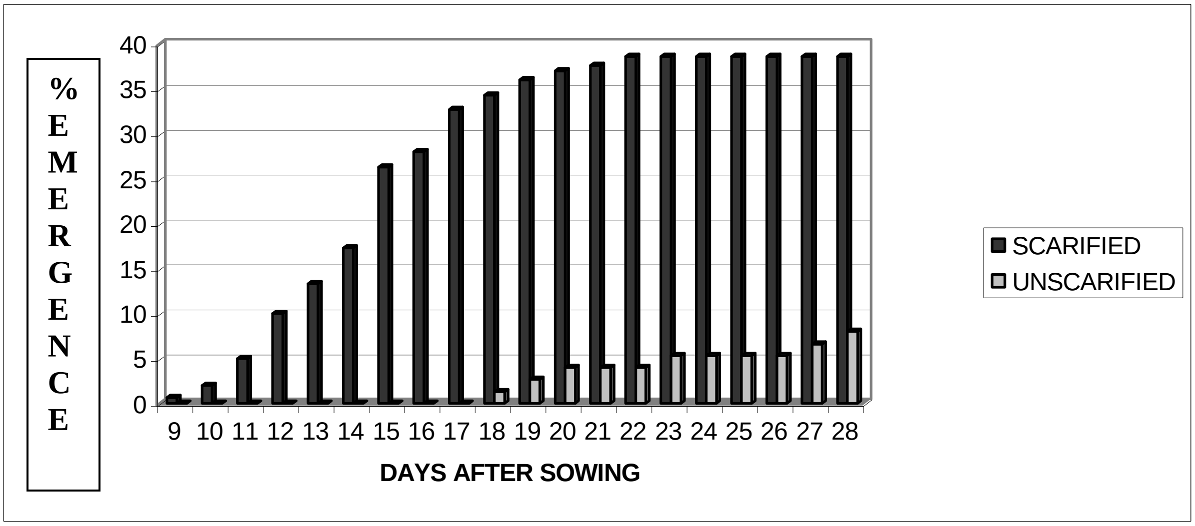

Fig. 2. Seedling Emergence from unscarified and chemically scarified (Conc. H_2SO_4 for 2 minutes) seeds of *Opuntia ficus-indica.*

Fig. 3. 15-day old seedlings of *Opuntia ficus-indica*. Note that few seedlings, indicated by arrows, had three cotyledons instead of usually two cotyledons. In all, 7.86% seedlings showed tricotyly.

The growth of seedlings is slow – relatively slower in tricotyledonous seedlings. In dicotyledonous seedlings major spurt in expansion of both large and small cotyledons of the seedling took place from 4 to 6 days after emergence (Fig. 5) – later the curve is more or less asymptotic. In case of tricotyledonous seedlings expansion of the large, medium and small cotyledons appeared to be somewhat slow but continuous as observed during initial 15 days of growth (Fig.6).

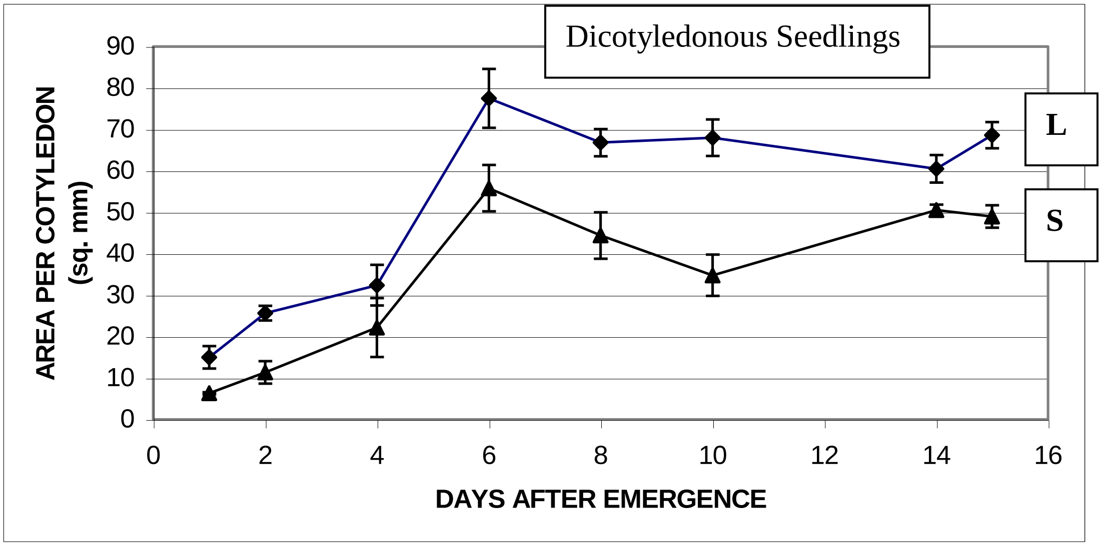

Fig. 5. Area of large (L) and small (S) cotyledons as a function of time in dicotyledonous seedlings of *Opuntia*.

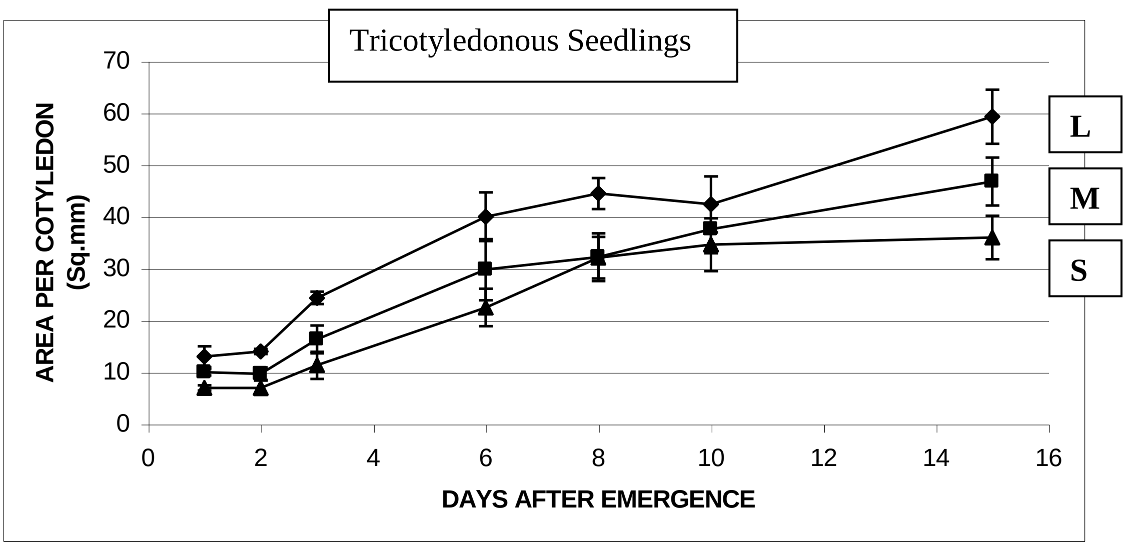

Fig.6. Area of large (L), medium (M) and small (S) cotyledons of tricotyledonous seedlings of *Opuntia* as a function of time.

The differences in cotyledonary area per seedling in two types of seedlings were generally insignificant except 3- and 15- day old seedlings. On 15-day seedling growth (Table 1), the cotyledonary area per seedling was 118.30 ± 5.46 mm^2 in dicotyledonous seedlings and 142.11 ± 13.28 mm^2 in tricotyledonous seedlings – only marginally different from one another ($p < 0.10$), which may signify little advantage to tricotyledonous seedlings on the basis of photosynthetic area. The two types of seedlings at this age didn't differ significantly from one another with respect to such morphometric parameters as radicle and hypocotyl lengths and thickness of hypocotyl. On 2.5 months of age dicotyledonous seedlings were 3.8 ± 0.24 cm in height including phylloclade - 1.94 ± 0.23 cm long bearing 17.8 ± 2.33 nodes. Tricotyledonous seedlings at this age were comparative in size (Height: 3.15 ± 0.25 cm including phylloclade 1.38 ± 0.23 cm long bearing 16.50 ± 2.66 nodes). Around this time cotyledons began turning brown and shrinking and then abscising.

The low germination percentage of seeds and phenotypic and genotypic variation of seedlings make this provenance of *Opuntia ficus-indica* less suitable for propagation by seeds. For genetic and economic reasons it may better be propagated vegetatively. Moreover, the species needs to be explored for its genetic peculiarities.

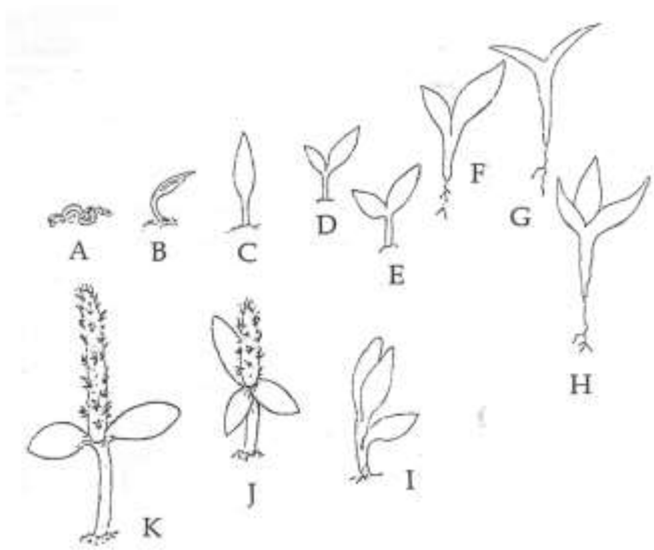

Fig.4. Various aged seedlings of *Opuntia ficus-indica*. A: hypocotyl emerging from soil; B: hypocotyl on emergence is bent hood of snake and generally bears two unequal cotyledons; C: hypocotyl takes around few hours to become erect while cotyledons still closely pressed; D & E: cotyledons open on seconds day of emergence; F & G: fifteen day old seedlings as seen from different angles; H: fifteen day old tricotyledonous seedlings; I: A 15 day old tricotyledonous seedling bearing a cotyledon abnormally – inserted nearly midway on the hypocotyls; J & K: nearly 2.5 months old dicotylendonous and tricotyledonous seedlings bearing phylloclade.

Table 1. Cotyledonary expansion in dicotyledonous and tricotyledonous seedlings.

Day After Emergence	**COTYLEDONARY AREA PER SEEDLING (mm^2)**	
	Dicotylednous Seedlings	**Tricotyledonous Seedlings**
1	21.33 ± 2.96; 4*, 24.06**	27.90 ± 2.00; 3, 26.31, NS ***
2	37.00 ± 4.36; 3; 20.40	30.67 ± 3.84; 3; 21.72, NS
3	39.05 ± 3.99; 4; 21.55	52.00 ± 4.51; 3; 15.02, $p < 0.10$
4	54.60 ± 11.50; 5; 47.09	-
6	128.25 ± 10.87; 4; 16.96	91.50 ± 13.81; 5; 30.19, NS
8	111.20 ± 5.99; 5; 12.06	108.00 ± 14.90; 4; 25.22, NS
10	102.60 ± 18.92; 4; 18.92	114.60 ± 14.59; 5; 28.47, NS
15	118.30 ± 5.46; 20; 20.63	142.11 ± 13.28; 9; 28.03, $p < 0.10$

*, N; **, Coefficient of Variability (%), ***, NS, Non-significant (t- test).

Table 2. Growth of 15-day old di- and tricotyledonous seedlings of *Opuntia ficus-indica*

Growth Parameter	**Dicotyledonous Seedlings (N 20)**	**Tricotyledonous seedlings (N=9*)**
Radicle (cm)	1.25 ± 0.108 CV: 38.80	1.00 ± 0.17 (N = 4), NS CV: 33.66
Hypocotyl length (cm)	2.19 ± 0.144 CV: 29.44	1.92 ± 0.25, NS CV: 38.40
Hypocotyl diameter (cm)	0.18 ± 0.004 CV: 10.36	0.21 ± 0.022, NS CV: 31.45

*, Out of 11, two small tricotyledonous seedlings died after 10 days of growth; NS, Non-significant by t-test even at $p < 0.10$.

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