Trakia Journal of Sciences, Vol. 7 , No. 1 , pp 12-16, 2009

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

Original Contribution

POLLEN FERTILITY (VIABILITY) STATUS IN ASTERACEAE SPECIES OF PAKISTAN

S. J. Qureshi[1*], M. A. Khan[2], M. Arshad[3], A. Rashid[4], M. Ahmad[5]

[1]Pakistan Islamia Institute AlAin, Abu Dhabi, United Arab Emirates;
[2]Department of Plant Sciences, Faculty of Biological Sciences, Quaid-i-Azam, University Islamabad, Pakistan;
[3]Department of Botany, PMAS Arid Agriculture University Rawalpindi, Pakistan;
[4]Department of Environmental Sciences, PMAS Arid Agriculture University, Rawalpindi, Pakistan;
[5]Physics Division, PINSTECH, P.O. Nilore Islamabad, Pakistan

SUMMARY

Pakistan is facing widespread destruction and degradation of its flora. This study was conducted to determine the pollen fertility status in forty five species of economic and environmentally important species, which reproduced freely and thus most suitable for rehabilitation in Pakistan. Pollen fertility status in these species varied as low as 42.3 % in *Scorzonera ammophila* to as high as 100 % in *Crepis flexuosa*, *C. multicaulis*, *Hieracium virosum*, *Lactuca altaica*, *L. lessertiana*, *L. orientalis*, and *L. saligna*. It was interesting to note that species within same genus showed wide range of pollen viability. However, most of the species studied had high pollen viability especially in those species, which grew in hilly areas. It was observed that pollen fertility was low in small fragmented populations. This showed the effect of population size on reproduction. Larger size populations showed high percentage of pollen fertility.

Key words: Pollen fertility, Viability, Asteraceae, Pakistan.

INTRODUCTION

Pakistan is located in south Asia at the north of the Tropic of Cancer and lies between 23^0 to 37^0 north latitude and 61^0 to 76^0 east longitudes (1). It has an area of 796095 km^2 covering about 0.6 % of the total area of the world. The flora is extremely diverse comprising more or less 6000 species (2) which include cultivated or naturalized taxa also and are found in a variety of habitats from seashore and deserts to high mountainous areas. About 372 species of plants are endemic, mostly found in the northern and western mountainous regions of Pakistan. Various environmental factors of Pakistan are being introduced here as every one can affect the distribution, growth, development and life cycles which lead to the establishment of climax communities of plants. Pakistan also encounters widespread destruction and degradation of its flora. The clearing of forests for plantations and settlements has resulted in the loss of many species.

The Asteraceae family consists of approximately 25,000 species (3) included in over 1100 genera. These species frequently present herbaceous habits, although arboreous and voluble herbaceous habits also occur (4). The Asteraceae (Compositae, alternate name) with its approximately 1,620 genera and more than 23,600 species is the largest family of flowering plants (5). The family is distributed worldwide except for Antarctica but is especially diverse in the tropical and subtropical regions of North America, the Andes, eastern Brazil, southern Africa, the Mediterranean region, central Asia, and south-western China.

Pollen fertility is generally high in both diploids and tetraploids. For example in the diploid *Tradescantia occidentalis*, 94 % of pollen was fertile, while 89 % pollen fertility was observed in tetraploids (6). High fertility of tetraploids pollen was also observed in *Rhoeo discolor* (Sw.) Stear, a close relative of *Tradescantia*, where tetraploids produced

*** Correspondence to**: *Sohail Jamil Qureshi, Pakistan Islamia Institute AlAin, Abu Dhabi, United Arab Emirates, P.O.Box # 15778; Mobile: 00971506930626; E-Mail: sohailjamilqureshi@hotmail.com*

twice as much fertile pollen as diploids (7). Due to the effect of temperature on chromosome pairing, pollen fertility in some *Tradescantia* species may be higher in periods of warm weather than after a series of cold, wet days (6).

The frequent removal of species together with the lack of understanding of consequences and advantages of replanting led to serious coastal degradation and to the endangerment of a high proportion of species. Therefore there is urgent need for conservation measures to rehabilitate the land and restore plant communities. Besides human activity, other factors have also contributed to the degradation of Pakistan flora. Periodic natural disasters such as hurricanes and storms, although infrequent, caused enormous devastation and destruction to plants and animals (8-11). In October, 1972 Hurricane Bebe swept across the Rotuma Island, its strong winds and accompanying unusually high waves caused extensive damage to homes and vegetation (12).

Pollen, which is a carrier of male gametes, includes three domains that are different in their chemical composition, morphological structure and their physiological and biological significance (13). The three domains of pollen grain include exine, intine and nucleus. The complex exine structures of pollen are storage sites for carbohydrates, glyco-proteins, lipids, terpenoids and phenolics (14). The pollen nucleus is rich in chromatin material and viable pollen stains pink to deep red with aceto-carmine, while srerile (mostly shrivelled) pollen does not take any stain and thus remains almost white and transparent (15, 16). A viable or fertile pollen is one which, after landing on stigma of the same plant or other plants of the same variety or species, under normal conditions would start growing a pollen tube and finally discharge its male gametes in the embryo sac effecting fertilization.

According to Rigamoto and Tyagi, (17) pollen fertility, which can be determined using pollen viability tests *in-vitro* is very important in fruit and seed production in flowering plants. Therefore, pollen fertility knowledge for any plant species is essential for plant breeders and commercial growers. They determine pollen fertility status using pollen viability tests *in-vitro* by aceto-carmine staining technique in thirty two species of economic and environmental importance. This investigation helps in identifying coastal species, which are reproducing freely and are therefore most suitable for coastal reforestation and rehabilitation.

MATERIALS AND METHODS

Different species of six genera belonging to Asteraceae (which were in flower during the time of this study) were tested for pollen fertility status by using the aceto-carmine staining method. The aceto-carmine method of determining viability in different species of plants is comparable to other methods such as pollen germination method on artificial medium (18) and Flow Cytometry Reactivity (19).

Flower buds, flowers or inflorescences of these species were collected from Pakistan and where possible their pollen was analysed for viability within one of collection or kept in the fridge at 4^{o} C for analysis the next day. The method followed that closely used by Marutani (16). Using dissecting forceps, scalpel and a needle, anthers of various species were opened to allow extraction and subsequent transfer of pollen dust on to a microscopic glass slide in a drop of aceto-carmine stain. Mature anthers were crushed and pollen grains mixed thoroughly with the aceto-carmine stain. Cover slips were gently placed on to different slides for each species. The slides were than observed under a light microscope. For each plant species flowers were collected from at least three different plants. For each plant, three slides were prepared. For each slide ten randomly selected fields were observed under the 10 X objective (100 X magnification).

To determine pollen fertility, darkly stained pollen grains were recorded as fertile and viable, and unstained or very lightly stained ones were considered as sterile or non-viable. Pollen fertility was calculated by dividing the number of viable pollen grains by the total number of grains counted in the field of view and averaging them for all plants in that species. Pollen viability was expressed as percentage pollen fertility in each plant species. Standard error was calculated from original data to compare for significant differences among different plant species. Collections were made from different sites, given in the map (**Figure A**

RESULTS

Pollen fertility results obtained using the aceto-carmine method are presented on **Table 1**. Average pollen viability ranged from a low 42.3±2.3 for *Scorzonera ammophila* to 100 % for *Crepis flexuosa*, *C. multicaulis*, *Hieracium virosum*, *Lactuca altaica*, *L. lessertiana*, *L. orientalis* and *L. saligna*. Out of the 45 species analysed for their pollen viability, only one species (2.2 %), namely *Scorzonera ammophila* showed a pollen viability percentage lower than 50 %. Fifteen species (33.3 %) showed pollen viability of 50-80 %. The remaining twenty nine species (64.4 %) showed a pollen viability percentage of 80-100 %.

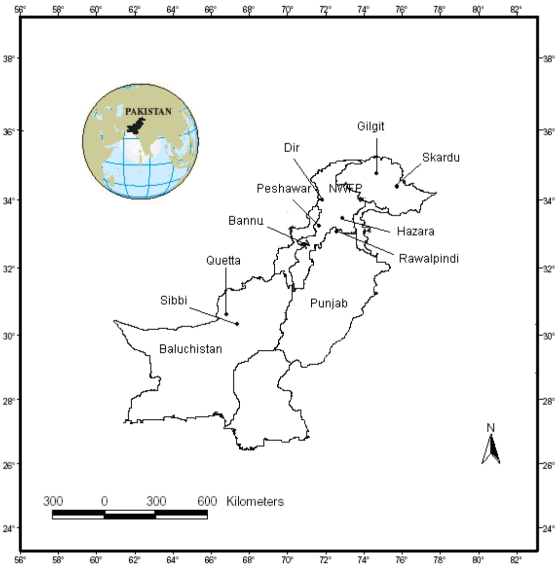

Figure A: *Location of sampling sites from Pakistan.*

Species within a single genus showed different pollen viability. For example in the *Lactuca* genus, *L. altaica* has an average pollen viability of 100 %, ± 0.0 with a range of 100-100 % while the least average value of pollen viability in *L. dissecta* is 52.6 %. The seven species of different genera, namely *Crepis flexuosa*, *C. multicaulis*, *Hieracium virosum*, *Lactuca altaica*, *L. lessertiana*, *L. orientalis*, and *L. saligna* showed 100 %, ±0.0 pollen viability. However, a larger difference was observed in different species of *Lactuca* genus. On the other hand, the seven species in pairs have same average pollen viability i.e. 69.3 % (*Lactuca serriola* L. f. *integrifolia* and *Sonchus arvensis*), 72 % (*Hieracium umbellatum* and *Lactuca brevirostris*), 78 % (*Scorzonera laciniata* and *Sonchus asper*), 78.3 % (*Scorzonera virgata* and *Sonchus palustris*), 80.3 % (*Sonchus oleraceus* and *Taraxacum aff* var. *glabrescence*), 93.3 % (*Taraxacum eriopodum* and *Taraxacum aff.* var. *typica*), 94.6 % (*Hieracium vulgatum* and *Tragopogon dubius*).

DISCUSSION

On **Table 1**, the pollen fertility (viability) status of most species tested was high. Several factors contributed to the low number of species included in this pollen fertility count. Theoretically it was possible to analyse all plant species. However, due to several factors it was not possible during the span of this study. Firstly, even though some species were in flower, they were usually too tall for easy flower collection. Secondly, most of the species were in the fruiting season at the time of this survey. Thirdly, the flowers of species were typically small having microscopic anthers. This made the extraction, crushing and counting of viable pollen grains a slow and tedious process. However, most of the species were analysed for their pollen viability.

In view of the impoverished flora of Pakistan, and the urgent need to reforest and replenish it, knowledge of the pollen fertility status of Pakistan plant species would be very useful. Pollen fertility is a significant determinant of whether in a population there will be enough regeneration through sexual reproduction to ensure the survival of that species. Pollen fertility is also evidently reduced in small fragmented populations (20). Other workers have investigated the effect of population size on reproduction of some angiosperms and found that reproduction was strongly reduced in small population where plants produced fewer seeds per fruit per plant (21-23). They concluded that insufficient quantity and quality of pollen was the likely explanation for the reduced fecundity in small populations. Fischer and Mathies (24) observed that fecundity was four times higher in large populations of the rare *Gentianella germanica* than in small populations and that fitness was reduced in plants from small populations. Menges (25) and Agren (26) also observed low pollen fertility and reduced seed germination rates in small fragmented populations.

Seed formation or regeneration information is not available; thus the main emphasis of this study was the concern that plants with low pollen fertility might become scarcer and could become endangered. Pollen fertility status of the plant species from Pakistan is required further investigation to include all the species. This will help to identify all the species with low pollen fertility, which would need replenishing.

Table 1: *Pollen fertility (viability) percentage in 45 Asteraceae plant species of Pakistan*

Scientific Names	***Pollen Fertility Percentage***	
	Range	*Mean ± S.E.*
Crepis flexuosa (Ledebour) C. B. Clarke	100-100	100±0.0
C. multicaulis Ledebour	100-100	100±0.0
C. sancta (L.) Babcock	74-80	77.3±2.0
C. stocksiana Aitch. & Hemsl	58-67	63.3±2.5
C. thomsoni Babcock	76-88	82.6±3.5
Hieracium bichlorophyllum (Druce & Zahn) Pugsley	63-79	73±4.1
H. diaphanoides Lindeb	90-95	92.6±2.3
H. umbellatum L.	68-75	72±1.7
H. virosum Pallas	100-100	100±0.0
H. vulgatum Fries	91-97	94.6±2.5
Lactuca altaica Fischer & C. A. Meyer	100-100	100±0.0
L. brevirostris Champ	67-75	72±2.0
L. clarkei Hooker	53-61	57.3±2.2
L. dissecta D. Don	48-56	52.6±2.4
L. dolichophylla Kitam.	47-59	54.3±3.2
L. graciliflora DC.	79-84	81±1.2
L. lessertiana C.B. Clarke	100-100	100±0.0
L. orientalis Boiss	100-100	100±0.0
L. remotiflora DC.	89-97	93.6±2.8
L. saligna L.	100-100	100±0.0
L. sativa L.	68-72	70±1.0
L. serriola L.	62-74	69±2.9
L. serriola L. f. *integrifolia* (Gray) S. D. Prince & R. N. Carter	66-72	69.3±1.9
L. tatarica C.A. Mey	77-86	82±2.1
L. undulata Ledeb	61-68	65±1.7
L. virosa L.	72-84	79.3±4.0
Scorzonera ammophila Bunge	38-47	42.3±2.3
S. hondae Kitam	53-61	57.6±2.5
S. laciniata L.	71-83	78±3.0
S. picridioides Boiss	78-89	84±2.6
S. virgata DC.	75-81	78.3±2.0
Sonchus arvensis L.	66-72	69.3±2.0
S. asper (L.) Hill	74-81	78±1.7
S. maritimus L.	88-95	92±1.7
S. oleraceus L.	75-84	80.3±2.5
S. palustris L.	76-80	78.3±1.6
S. uliginosus M. Bieb.	58-65	62±1.7
Taraxacum eriopodum (D. Don) DC.	87-99	93.3±3.2
T. aff var. *glabrescence* Web.	77-84	80.3±2.1
T. glaucescens Wigg	68-87	76.3±4.7
T. laevigatum (Willd.) DC	74-79	76.6±2.2
T. aff. var. *parvula* Wigg and Boiss	81-97	90±3.8
T. aff. var. *typica* Hk	89-97	93.3±2.4
Tragopogon dubius Scop	88-99	94.6±3.4
T. gracilis D. Don	76-90	83.6±3.8

ACKNOWLEDGEMENT

We are grateful to Chairman, Department of Plant Sciences, Faculty of Biological Sciences Quaid-i-Azam University Islamabad, for his assistance in the herbarium and palynological lab. He also made constructive comments on an early version of the manuscript.

REFERENCES

1. Horsburgh N. Oxford social studies for Pakistan, Book. 5, ed.8. Oxford University Press Karachi, 1998, p. 26.
2. Stewart RR. Annotated catalogue of the vascular plants of the west and Kashmir. Fakhri Printing Press, Karachi, 1972.
3. Barroso GM. Sistematica de Angiospermas do Brasil. Vicosa: Universidade Federal de Vicosa, 1986.
4. Cronquist A. An Integrated System of Classification of Flowering Plants. Columbia: University Press, 1981.
5. Stevens PF. Angiosperm Phylogeny Website. Version 8, June 2007 [and more or less continuously updated since]. http://www.mobot.org/MOBOT/research/APweb/. 2001.
6. Anderson E, Sax K. Cytological monograph of North American species of *Tradescantia*. Bot. Gaz. 1936; 97:433-476.
7. Walters MS, Gerstel DU. A cytological investigation of a tetraploid *Rhoeo discolor*. American Journal of Botany 1948; 26:141-150.
8. Dumont d' Urville JSC. Voyage Pittoresque Autour du Monde; Resume General des Voyages de De Couvertes Public sons la Directions de M Dumouint d' Urville Accompagne de Cartes et de Nombreuses Gravures En Tailledouce sur Acir, d'apress les Dessins de M. de Sainson, Dessinateur du Voyage de l' Astrolobe. Furne et cie 1848; 41:460-464. (English Translation)
9. Boddam-Whetham JW. Rotuma. In: *Pearls of the Pacific*. Hurst and Blackett, London, 1876; pp.266-273.
10. Gardiner JS. The natives of Rotuma. Journal of Royal Anthropological Institute 1898; 27:457-459.
11. Bach S. Rotuma: A dependency of Fiji. *The colony of Fiji*: 1874-1924. Government Printer, Suva,1924; pp.154-157.
12. Walkley B. Rotuma, where giants lived and dead sleep in luxury. Pacific Island Monthly 1973; 44:47-49.
13. Knox RB. Cellular Interaction. Plant Physiology 1984; 17:508-520.
14. Weirmann R, Gubatx S. Pollen Wall and Sporopollenium. In International Review of cytology- sexual reproduction in plants. Harcourt Brace Jovanovich Publishers, London, 1992; pp.508-608.
15. McKellar MA, Quesenberry KH. Chromosome pairing and pollen viability in *Desmodium ovalifolium* Wall *x Desmodium heterocarpon* (L.) DC hybrids. Australian Journal of Botany 1992; 40:243-247.
16. Marutani M, Sheffer RD, Kameto H. Cytological analysis of *Arithurium andraenum* (Araceae), its related taxa and their hybrids. American Journal of Botany 1993; 80:93-103.
17. Rigamoto RR, Tyagi AP. Pollen fertility status in coastal plant species of Rotuma Island. South Pacific Journal of Natural Science 2002; 20:30-33.
18. Tyagi AP, Considine J, McComb J. Germination of *Verticordia* pollen after storage at different temperatures. Australian Journal of Botany 1992; 40:151-155.
19. Tyagi AP, Dass CR, Nathan R, Racule T, Lakhan S. Pollen fertility status in some flowering plant species of Fiji. South Pacific Journal of Natural Science 1995; 14:211-222.
20. Jennersten O, Nelssen SG. Insect flower visitation frequency and seed production in relation to patch size in *Viscara vulgaris* (caryophyllaceae). Oikos 1993; 68:283-292.
21. Byers DL. Pollen quantity and quality as explanation for low seed set in small populations exemplified by *Eupatorium* (Asteraceae). American Journal of Botany 1995; 82:1000-1006.
22. Kerry M. The effect of population size on reproduction of the grassland species *Gentiano lutia* and *Primula veris L*. Diploma Thesis, University of Basel, Basel, Switzerland, 1995.
23. Kerry M, Matthies D, Spillman HH. Reduced fertility and offspring performance in small populations of the declining grassland plants *Primula veris* and *Gentiana lutea*. Journal of Ecology 2000; 88:17-30.
24. Fischer JE, Matthies D. Effects of Population size on performance in the rare plant *Gentianella germinica*. Journal of Ecology 1998; 86:195-204.
25. Menges BS. Seed germination percentage increases with population size in a fragmented prairie species. Conservation Biology 1991; 5:158-164.
26. Agren J. Population size, pollinator limitation and seed set in the self-compatible herb *Lythrum salicaria.* Ecology 1996; 77:1779-1790.