Weed Seeds Intercepted in *Trifolium* spp. Germplasm Imported from USA and Egypt

Mool Chand Singh, B Lal, PC Agarwal, Anjula Pandey, KD Joshi, Dinesh Chand and RK Khetarpal

National Bureau of Plant Genetic Resources, New Delhi-110012, India

A total of eighty three seed samples of red clover (*Trifolium pretense* L.) and berseem clover (*T. alexandrinum* L.) germplasm were imported from USA and Egypt, respectively through National Bureau of Plant Genetic Resources, New Delhi during 2007. While processing the consignment for quarantine clearance, a total of 17 species of weeds were intercepted, of which nine species are not yet reported from India, whereas, eight species are known to occur in different parts of the country. Five exotic weed species had a Weed Risk Assessment (WRA) score of more than 12 indicating their potential to become serious weed and fifteen weed species were found viable even after two and half years of storage at room temperature. The observations indicated that import of red clover and berseem clover seeds from USA and Egypt has the risk of introduction and dissemination of exotic weeds into India. The weed species intercepted, their weed potential and seriousness using Weed Risk Assessment (WRA) system are discussed.

Key Words: *Trifolium*, Plant Quarantine, Exotic Weeds, Weed Risk Assessment

Introduction

Plant Genetic Resources in the form of seeds or vegetative propagules etc. are regularly imported for research and crop improvement programmes in our country. However, such imports also lead to the introduction of weeds and other pests and thus, pose quarantine risk. Increasing trade and globalization coupled with liberalized policies further increase the risk of introduction of exotic weeds through bulk imports. This could lead to decrease in native biodiversity, reduced productivity of different ecosystems, reduced input-use efficiency and increased production cost. It is estimated that one-third of oilseeds, half of the food grains and an equal amount of pulses produced currently are lost due to weeds. The potential yield losses due to weeds could be as high as 65 per cent depending upon the crop, type of weed species, degree of weed infestation, and management practices (Yaduraju *et al.*, 2006). Some of the major alien invasive weeds that have entered into the country along with the movement of plant material etc. include *Chromolaena odorata* L., *Eichhornia crassipes* (Mart.) Solms, *Lantana camara* L., *Mikania micrantha* H.B.K., *Mimosa* spp., *Parthenium hysterophorus* L., *Salvinia molesta* D.S. Mitchell, etc. (Yaduraju *et al.*, 2003).The environmental and socio-economic impacts of harmful invasive alien plant species are evident in case of alien aquatic weeds like water hyacinth (*E. crassipes*), water lettuce (*Pistia stratiotes* L.) and terrestrial weeds such as *L. camara*, *P. hysterophorus* and *Phalaris minor* Retz. (Rana *et al.*, 2003). The Central Government had imported nearly 63 lakh metric tones of wheat during 2006-07 from different countries in which 25 weed species were intercepted. The National Invasive Weed Surveillance (NIWS) team has traced five invasive weeds which came to India through this wheat import (Joseph John, 2009).

National Bureau of Plant Genetic Resources (NBPGR), New Delhi is the nodal agency that facilitates exchange of plant germplasm meant for research between India and different countries. It has the power vested by the Plant Protection Adviser to the Government of India, under the Plant Quarantine (Regulation of Import into India) Order 2003, of the Destructive Insects and Pests Act (1914) to carry out quarantine of the plant germplasm including transgenic imported for research purpose. In order to ensure effective implementation of Plant Quarantine (Regulation of Import into India) Order 2003, it is essential that all imported seed samples are free from weeds of quarantine importance. Therefore, all samples of red clover and berseem clover were examined for the presence of weed seeds especially to determine the presence of quarantine weeds. The present study highlights the interception of weeds from imported germplasm of red clover and berseem clover from USA and Egypt and assessment of their potential risk through Weed Risk Assessment (WRA) system.

Materials and Methods

A total of 83 samples of red clover (77) from USA and berseem clover (6) from Egypt were processed at NBPGR for quarantine clearance during 2007. All seed samples were visually examined under higher magnification for detection of weed seeds. Each sample was spread in a thin uniform layer on a clean white drawing sheet and examined under illuminated magnifier (IMG50 with

magnification 6X) for separating the weed seeds. They were segregated into different types on the basis of their shape, size, colour, texture, etc. and observed under stereoscopic binocular (Wild (Heerbrugg) M5-86173, magnification 10X).Identification of weed seeds was done upto species level based on their morphological characters using Identification Keys (Anonymous, 1998) and with the help of Weed Seed Identification Kit developed by Academy of Grain Technology, Australia (Anonymous, 1997). Three weed species namely *Amaranthus retroflexus* L., *Chenopodium album* L. and *Panicum texanum* Buckl. were subjected to grow out test in glass house in isolation and identified on the basis of their vegetative characters (Nayar and Pandey, 2009) and subsequently plants were incinerated. All weed seeds were tested for their viability by wet blotter method in *Petri* dishes under strict quarantine conditions in incubation room at 21^0C temperature and germination was counted on eighth day. The weed species were also assessed through Weed Risk Assessment (WRA) system for their weed potential and seriousness (Moolchand *et al*., 2001). Weed Risk Assessment is a question based scoring system, containing 49 questions about the species. The questions include details of the plant's climatic preferences, biological attributes, reproduction and dispersal methods (Table 2). A minimum number of questions must be answered before an assessment is made. The WRA uses responses to the questions to generate a numerical score that is positively correlated with weediness. The plants which have score between 0-6 are non weeds, 7-11 are common weeds and those having >12 score, are serious weeds (Groves *et al*., 2001).

Results and Discussion

The results indicated that out of 77 samples of *T. pretense* imported from USA, 26 samples were found to be contaminated with 12 weed species. Similarly out of six samples of *T. alexandrinum* imported from Egypt, all samples were found to be contaminated with five weed species. Weed species intercepted from USA included *Amaranthus retroflexus* L., *Carrichtera annua* L., *Chenopodium album* L., *Ipomoea hederacea* L. (Jacq.), *Medicago sativa* L., *Panicum texanum* Buckl., *Phalaris paradoxa* L., *Polygonum convolvulus* L., *P. hydropiper* L., *P. persicaria* L., *Trifolium microcephalum* L. and *T. variegatum* L. while those from Egypt included *Cichorium intybus* L., *C. spinosum* L., *Rumex crispus* L., *R. pulcher* L. and *R. solicifolius* Weinm. (Fig. 1). Among 17 weed species intercepted, nine species such as *C. annua, P. paradoxa, P. persicaria, T. microcephalum, T. variegatum, C. spinosum, R. crispus R. pulcher* and

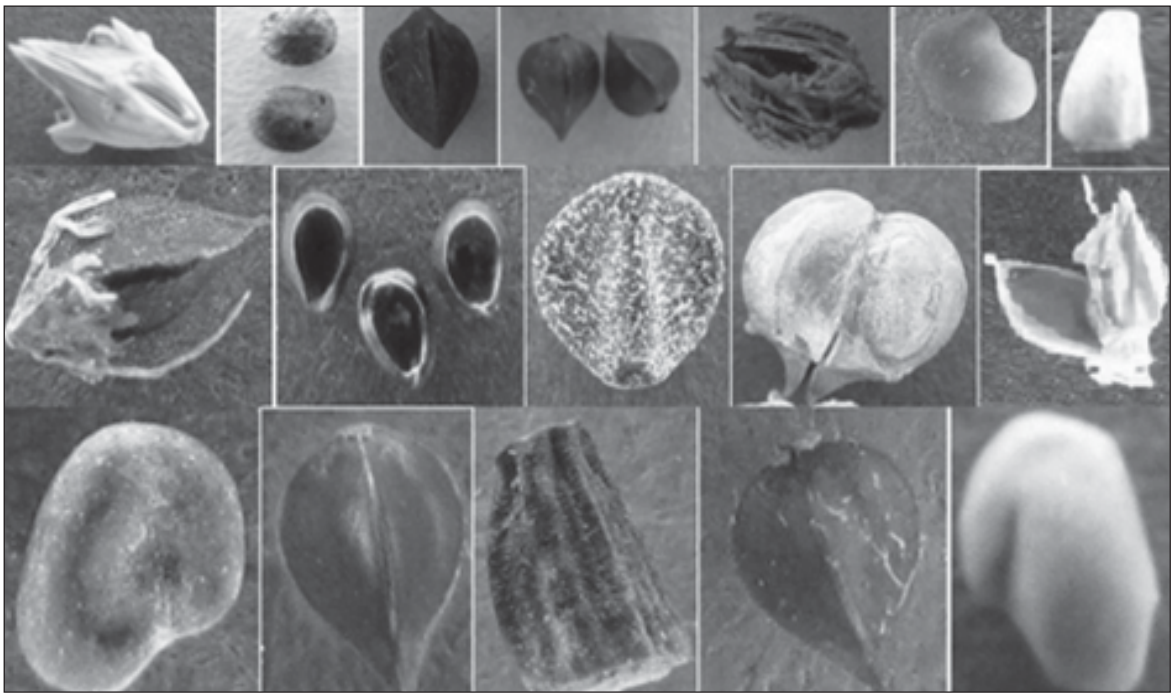

Fig. 1: Weed species intercepted: (First row from left to right) *Phalaris paradoxa, Chenopodium album, Polygonum persicaria, Rumex pulcher, Polygonum convolvulus, Trifolium microcephalum, Cichorium intybus* (second row from left to right) *Polygonum hydropiper, Amaranthus retroflexus, Ipomoea hederacea, Carrichtera annua, Panicum texanum,* and (third row from left to right) *Trifolium variegatum, Rumex crispus, Cichorium spinosum, Rumex solicifolius* and *Medicago sativa*.

R. solicifolius are not yet reported from India whereas, eight species occurr in different parts of the country (Holm *et al*., 1979). Fifteen weed species were found viable even after long storage of seeds. Five of these had a WRA score of >12 indicating their high potential to become serious weed in agriculture(Groves *et al*., 2001). Out of 12 weed species intercepted in red clover seeds from USA, six species namely *A. retroflexus, C.album, P. paradoxa, P. convolvulus, P. hydropiper* and *P. persicaria* have developed multiple resistance against herbicides in USA (Moss, 2002).If these weeds are introduced into India, they may cause havoc to Indian agriculture. Our farmers are already struggling with *Phalaris minor* weed in wheat crop as it has developed resistance against isoproturon herbicide (Malik and Singh, 1995).Gradual decrease in the efficiency with the development of resistance have turned into deeper problem resulting in crop failure at many farms (Malik, 1995). Bhan and Williams (2006) reported that grain yield of wheat was reduced by 14.8 % with *P. minor* and 17.4% in competition with *P. paradoxa*. This indicates that *P. paradoxa* is a more aggressive competitor with wheat than *P. minor*. Weed seeds identified in the imported germplasm, their viability and WRA score are given in Table 1.

The observations indicated that import of *T. pratense* and *T. alexandrinum* seeds could be source of introduction of exotic weeds into India. The WRA scores reveal that 14 species have potential to become serious weed in agriculture. It is evident that seeds of fifteen weed species were viable even after two and half years of storage at normal temperature indicating their ability to grow and spread under field conditions. All the weed seeds were removed from *T. pratense* and *T. alexandrinum* seeds by mechanical cleaning and only weed free samples were released and made available to the importer.

In order to prevent introduction of weeds, particularly the ones that are problematic in related countries need to be subjected to Weed Risk Assessment. Weeds seeds in imported samples could be serious threat to the country if not detected. Relatively unknown potential of these spreading to larger areas may turn to noxious and warrant study of their effect on the ecosystems besides effect on human and animal health. Critical quarantine examination of all the imported seeds is necessary to check the introduction of new weeds into the country. There is also an urgent need to design safeguards and strengthening of quarantine regulations to lower the risk of their entry.

Table 1. Weed seeds intercepted, number and %age of contaminated samples, germination (%) and Weed Risk Assessment (WRA) score

Weed species intercepted	Common name	Family	Average contamination % (Number basis)	No. of contaminated samples	% of contaminated samples	Germi-nation (%)	WRA score
Red clover seeds from USA							
Amaranthus retroflexus	Pigweed	Amaranthaceae	02	11	14.28	45	07
*Carrichtera annua**	Wards weed	Brassicaceae	03	06	07.79	62	08
Chenopodium album	Lambsquarters	Chenopodiaceae	04	14	18.18	00	06
Ipomoea hederacea	Ivyleaf morning-glory	Convolvulaceae	01	05	06.49	40	13
Medicago sativa	Alfalfa	Papilionaceae	02	03	03.89	72	00
Panicum texanum	Texas millet	Poaceae	01	07	09.10	82	13
*Phalaris paradoxa**	Hood canarygrass	Poaceae	01	10	12.98	32	14
Polygonum convolvulus	Black bindweed	Polygonaceae	02	08	10.38	26	10
P. hydropiper	Common smartweed	Polygonaceae	02	12	15.58	28	09
*P. persicaria**	Ladysthumb	Polygonaceae	01	04	05.19	30	08
*Trifolium microcephalum**	Big-flowered clover	Papilionaceae	01	03	03.89	68	06
*T. variegatum**	Whitetip clover	Papilionaceae	01	05	06.49	59	08
Berseem clover seeds from Egypt							
Cichorium intybus	Chicory	Asteraceae	06	06	100.00	80	07
*C. spinosum**	Spiny chicory	Asteraceae	03	06	100.00	74	15
*Rumex crispus**	Curly dock	Polygonaceae	02	06	100.00	00	14
*R. pulcher**	Fiddleleaf dock	Polygonaceae	03	05	83.33	20	08
*R. solicifolius**	Willow-leaved dock	Polygonaceae	02	04	66.66	32	09

* Weeds not yet reported from India

Table 2. Specimen of Weed Risk Assessment (WRA) question sheet

Answer yes (y) or no (n), or don't know (leave blank or ?), unless otherwise indicated

	Botanical name: Common name: Family name			Outcome: Score: Your name:
				History/Biogeography
A	1	*Domestication/ cultivation*	1.01	Is the species highly domesticated? If answer is 'no' go to question 2.01
C			1.02	Has the species become naturalized where grown
C			1.03	Does the species have weedy races
	2	*Climate and distribution*	2.01	Species suited to Indian climates (0-low; 1-intermediate; 2-high)
			2.02	Quality of climate match data (0-low; 1-intermediate; 2-high)
C			2.03	Broad climate suitability
C			2.04	Native or naturalized in regions with extended dry periods
			2.05	Does the species have a history of repeated introductions outside its natural range
C	3	*Weed elsewhere*	3.01	Naturalized beyond native range
E			3.02	Garden/amenity/disturbance weed
A			3.03	Weed of agriculture/horticulture/forestry
E			3.04	Environmental weed
			3.05	Congeneric weed
				Biology/Ecology
A	4	*Undesirable traits*	4.01	Produces spines, thorns or burrs
C			4.02	Allelopathic
C			4.03	Parasitic
A			4.04	Unpalatable to grazing animals
C			4.05	Toxic to animals
C			4.06	Host for recognized pests and pathogens
C			4.07	Causes allergies or is otherwise toxic to humans
E			4.08	Creates a fire hazard in natural ecosystems
E			4.09	Is a shade tolerant plant at some stage of its life cycle
E			4.10	Grows on infertile soils
E			4.11	Climbing or smothering growth habit
E			4.12	Forms dense thickets
E	5	*Plant type*	5.01	Aquatic
C			5.02	Grass
E			5.03	Nitrogen fixing plant
C			5.04	Geophyte
C	6	*Reproduction*	6.01	Evidence of substantial reproductive failure in native habitat
C			6.02	Produces viable seed
C			6.03	Hybridises naturally
C			6.04	Self-fertilisation
C			6.05	Requires specialist pollinators
C			6.06	Reproduction by vegetative propagation
C			6.07	Minimum generative time (years)
A	7	*Dispersal mechanisms*	7.01	Propagules likely to be dispersed unintentionally
C			7.02	Propagules dispersed intentionally by people
A			7.03	Propagules likely to disperse as a produce contaminant
C			7.04	Propagules adapted to wind dispersal
E			7.05	Propagules have dormancy
E			7.06	Propagules bird dispersed
C			7.07	Propagules dispersed by other animals (externally)
C			7.08	Propagules dispersed by other animals (internally)
C	8	*Persistence attributes*	8.01	Prolific seed production
A			8.02	Evidence that a persistent propagule bank is formed (>1 yr)
A			8.03	Well controlled by herbicides
C			8.04	Tolerates or benefits from mutilation, cultivation or fire
E			8.05	Effective natural enemies present in India

A= agricultural, E = environmental, C= combined

Source: Modified from Australian WRA Model (Groves *et al*., 2001)

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References

Anonymous (1998) Weed Seed Identification Guide. Southern Weed Science Society, USA

Anonymous (1997) Weed Seed Identification Kit. Academy of Grain Technology. Australian Wheat Board, Victoria, Australia.

Bhan A and Williams RJ (2006) *Phalaris* spp. competition with wheat using an additive design series. Proceedings of 15th Australian weeds conference, 24-28 September, Adelaide, Australia, pp. 417-19.

Groves RH, FD Panetta and JG Virtue (2001) Weed Risk Assessment. CSIRO Publishing, 150, Oxford street, P O Box 1139, Collingwood, VIC 3066, Australia.

Holm L, JV Pancho, JP Herberger and DL Plucknett (1979) A Geographical Atlas of World Weeds. John Wiley & Sons, New York.

Joseph John (2009) Wheat import mixed blessing, hunt on for 'invasive' weeds. Indian Express, September 25, 2009.

Malik RK and S Singh (1995) Little seed canary grass (*Phalaris minor)* resistance to isoproturon in India. *Weed Technol.* **9:** 419-425.

Malik RK (1995) Herbicide resistance and its management against *Phalaris minor* in India. Weed News **2(1&2):** 39-40.

Moolchand PA, Wiilliams, C Walton and SW Adkins (2001) Weed Risk Assessment system for India. In proceedings Biennial Conference on Weed Science, May 23-25, 2001. University of Agricultural Sciences, Bangalore, India.

Moss SR (2002) Herbicide-Resistant weeds. Weed Management Hand Book (Robert EL Naylor (eds.).Wiley Interscience Inc. New York, USA.

Nayar ER and A Pandey (2009) Detection and identification of weeds in quarantine. In: Training Manual on Biosecurity and Biosafety Policies, Procedures and Issues (V Celia Chalam, K. Gupta, Shasi Bhalla,, Rajan and RK Khetrapal (eds.). Pp 236-246. January 14-February 3, National Bureau of Plant Genetic Resources, New Delhi.

PQ Order (2003) Plant Quarantine (Regulation of Import into India) Order (2003). Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India.

Rana RS, BS Dhillon and RK Khetarpal (2003) Invasive alien species: The Indian scene. *Indian J Plant Genet. Resour*.**16**: 190-213.

Yaduraju NT, MBBP Babu and AK Gogoi (2003) Green Invaders- A growing threat to agriculture and environment. In Proceedings of National Seminar on Alien Invasive Weeds in India, pp. 1-9. April 27-29, 2003, AAU, Jorhat.

Yaduraju NT, MBBP Babu and Poonam Chandla (2006) Herbicide Use. In: Agriculture and Environment. Swaminathan, MS and Chadha, KL (eds). pp. 192-210. Malhotra Publishing House, New Delhi, India.