



Removal of tenacious glumes enhances the seed germination and seedling vigour in eight *Aegilops* species for efficient conservation in genebank

K SRINIVASAN¹, J RADHAMANI², T KALKANDE³ and R K TYAGI⁴

National Bureau of Plant Genetic Resources, New Delhi 110 012

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ABSTRACT

Wild species, which are potentially valuable sources of resistance, play a very prominent role as donors of stress resistant genes in crop improvement programmes. Thus, their conservation in the form of seeds in the genebanks is of paramount importance for their availability in breeding programmes. High initial seed quality is one of the important pre-requisites for long-term conservation in genebanks which is measured in terms of seed germination and vigour. *Aegilops* species exhibit inherent problems caused by erratic and staggered germination due to dormancy imposed by presence of tenacious glumes. Objective of this study was to evaluate the effects of removal of glume to assess the initial seed germination and seedling vigour of eight wild species of wheat before their long-term storage in genebank. Germination in seeds with glume was recorded as 20-70% and in seeds without glume was 72-100%. It is concluded that tenacious glumes contribute to the physical dormancy and removal of glumes increased the germination and seedling vigour in all eight species namely *Ae. bicornis*, *Ae. geniculata*, *Ae. kotschyi*, *Ae. longissima*, *Ae. triuncialis*, *Ae. umbellulata*, *Ae. vavilovii*, and *Ae. ventricosa*. We recommend that the genebank curators can follow this method to assess the initial seed viability, which is pre-requisite, for long-term conservation of valuable donor *Aegilops* species in genebank.

Key words: *Aegilops*, Conservation, Dormancy, Germination, Wheat, Wild species

Wild relatives of crop plants are very important component of the plant genetic resources and constitute a potential rich source of genetic variation (Feldman and Sears 1981). Despite the fact that their overall performance is agronomically less desirable, favorable alleles are present in wild relatives. Wild relatives have played significant role in developing new cultivars and have increased the productivity of globally important crops such as barley, maize, oats, potatoes, rice and wheat.

The genus *Aegilops* is closely related to *Triticum* (Kerby and Kuspira 1988, Haider *et al.* 2010). Due to the presence of potentially valuable traits, the wild species of wheat play a very prominent role as donors of abiotic and biotic stress tolerant genes for future crop improvement programmes (Monneveux *et al.* 2000 and references therein; Asseefa and Fehrman 2004). Future gains in yield potential, especially under stressed conditions, will almost certainly require exploitation of the largely untapped sources of

genetic diversity housed in collections of wheat landraces and wild relatives (Skovmand *et al.* 2001). Wild wheats and landraces, especially material adapted to micro-habitats, are rapidly disappearing because of the introduction of agronomically superior new cultivars. Thus, their conservation in the form of seeds in the genebanks is of paramount importance to safeguard the germplasm from potential threats such as climate change and these are the sources from which breeders seek the germplasm with specific traits for their availability in breeding programmes. For long-term conservation in genebanks (at -20°C), high initial seed quality is one of the important pre-requisites. This is ensured by conducting standard germination test in the laboratory following the ISTA Rules (1999) and selecting the accessions registering high germination potential which are further processed for moisture equilibration to recommended level and packed in special containers for long-term storage. While processing the germplasm of wild wheat, some species of *Aegilops* like other wild species of Poaceae exhibited erratic and staggered germination due to dormancy imposed by tough seed covering structures. Seeds of all the wild species exhibited poor and erratic germination when subjected to standard germination test before processing for long-term conservation. Since the seeds were

¹ Principal Scientist (e mail: kalyani@nbpgr.ernet.in), ² Senior Scientist (e mail: radhamani@nbpgr.ernet.in), ³ Former Research Associate (e mail: toshikalkande@yahoo.co.in), ⁴ Principal Scientist and Head (e mail: rktyagi@nbpgr.ernet.in, tyaginbpr@gmail.com), Division of Germplasm Conservation

freshly harvested and also remained fresh at the end of prolonged germination test period (about 30 days), it was presumed that the seeds were dormant. Seeds of all the species had their caryopsis enclosed by tenacious glumes which acted as physical barrier to water absorption and exchange of gases. It is essential to overcome this dormancy to assess the actual viability before long-term conservation. The study was conducted to see the effect of removal of tenacious glumes and the extent of improvement in the germinability, uniformity of germination and seedling vigour of eight *Aegilops* species, as no work on dormancy breaking methods are documented in literature on wild species except *Ae. kotschy* (Wurzbinger and Lesham 1969).

MATERIALS AND METHODS

Seeds of eight *Aegilops* species, viz. *Ae. bicornis* Jaub. & Spach., *Ae. geniculata* Fig. & De Not, *Ae. kotschy* Boiss., *Ae. longissima* Schweinf., Muschl. & Eig., *Ae. triuncialis* L., *Ae. umbellulata* Zhuk., *Ae. vavilovii* (Zhuk.) Chennav., and *Ae. ventricosa* Tausch, harvested in same season and climatic conditions at NBPGR Regional Station, Bhowali, were used for the present study. A total of 150 seeds in three replicates (50 seeds/replicate) for each species were used for the standard germination test (ISTA 1999). One set of seeds were manually stripped off of their glume (cupulated fruit cases) by using sharp surgical blade. Another set of seeds with glume was used as control. Care was taken to avoid injury to the pericarp as well as the embryo. Seeds were tested for germination following 'between paper' method at 25°C and evaluated on the seventh day. The rate of germination expressed as the mean germination time (MGT) was calculated using the formula: $MGT = \sum nd / \sum O_n$, where n = number of seeds which germinate on a particular day "d" and d = number of days counted from the start of germination test. A seed was considered to have germinated when 1 mm radical had emerged (Ellis and Roberts 1981).

To test the seedling vigour, owing to the limitation of seed quantity, 75 seeds in three replicates (25 seeds/replicate) were placed in a single line, equidistantly on paper towels which were rolled and arranged in polythene bags loosely to allow proper aeration and incubated in a seed germinator at 25°C in dark. On the 14th day of planting the seeds, the root, shoot and coleoptile length (cm) were measured for all the germinated seedlings and their sum divided by number of seeds germinated to express root, shoot or coleoptile vigour, respectively. Mean seedling vigour was calculated by adding the means of the three components. Seedling vigour index (VI) was calculated by the method of Abdul-Baki and Anderson (1973) as the product of germination (%) and seedling vigour of each replication. The 1000-seed weight (g) for all the eight species before and after the removal of glume was recorded. The results were subjected to statistical analysis and the critical difference was used to compare the means of all parameters studied.

RESULTS AND DISCUSSION

Seed germination and mean germination time

Germination started after 3-6 days in controls and 2-3 days in deglumed seeds in all the tested species considered together. Seeds of all the *Aegilops* species exhibited germination in controls ranging from 20-70%. The lowest germination (20%) was recorded in *Ae. vavilovii* and maximum (70%) in *Ae. ventricosa*. After degluming, the germination increased significantly up to five-fold, being maximum (up to 100%) in *Ae. bicornis* and *Ae. vavilovii* (Fig 1). Degluming increased the seed germination; seeds of all the species exhibited more than 65% germination after degluming. *Ae. longissima* did not show much improvement in seed germination (72%) as compared to control (66%) though it was significantly higher than control. Dormancy and germination are traits that are controlled in a highly complex manner involving one or a combination of morphology, physiology and physical structures (Baskin and Baskin 2001, Finch-Savage and Leubner-Metzger 2006). Seed dormancy is often caused by structures surrounding the embryo, physiological stage of the embryo, and its maturity, the permeability of the seed coat (Knapp 2000), as well as a combination of all these factors. The extent of the problem is variable and considerable differences occur between species, ecotypes, locations, and year of harvest (Laude 1956, Tian *et al.* 2002). Wurzbinger and Lesham (1969) have clearly brought out the germination inhibitory nature of extract of glume and hull of *Ae. kotschy*. Presence of germination inhibitors in the aqueous glume and hull extracts of certain poaceae species was reported by Miyamoto *et al.* (1961) and Waisal and Adler (1959). Gatford *et al.* (2002) have confirmed the presence of water soluble phenolic substances in the crude extracts of the bracts of *Triticum tauschii*. It is quite possible that dormancy was imposed by inhibitors present in

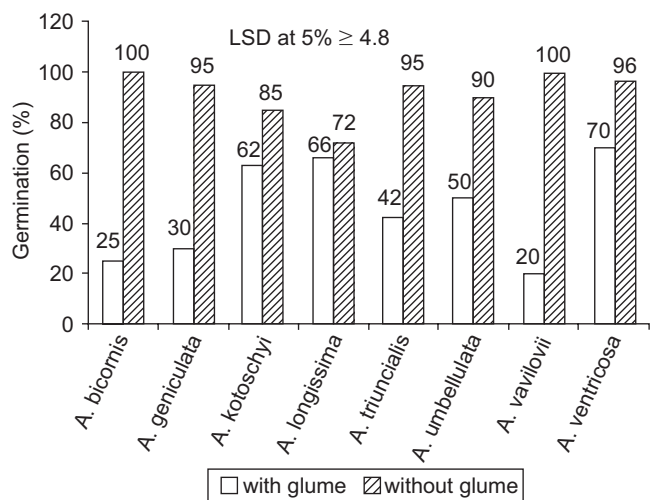


Fig 2 Effect of removal of glumes on the germination (%) of *Aegilops* species

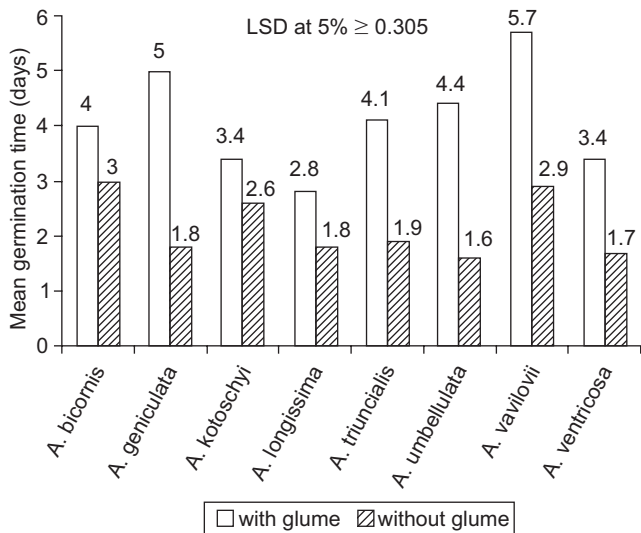


Fig 2 Effect of removal of glumes on the mean germination time (MGT) of *Aegilops* species

the covering structures in the *Aegilops* species considered for the present study, the removal of which released the dormancy completely or partially.

The MGT for control seeds was maximum in *Ae. vavilovii* followed by *Ae. geniculata* (5-6 days). The earliest and fastest germination was recorded as 2.8 days in the control seeds of *Ae. longissima* (Fig 2). Maximum and highly significant improvement in the MGT due to degluming was observed for *Ae. geniculata* (from 5 to 1.8 days) followed by *Ae. vavilovii* (from 5.7 to 2.9 days) and *Ae. umbellulata* (from 3.4 to 1.6 days) (Fig 2). Thus, degluming not only improved the germination but decreased the mean germination time also in all the species tested. Various factors such as control of water uptake, selective permeability to gases, presence of germination inhibitors in the covering structures,

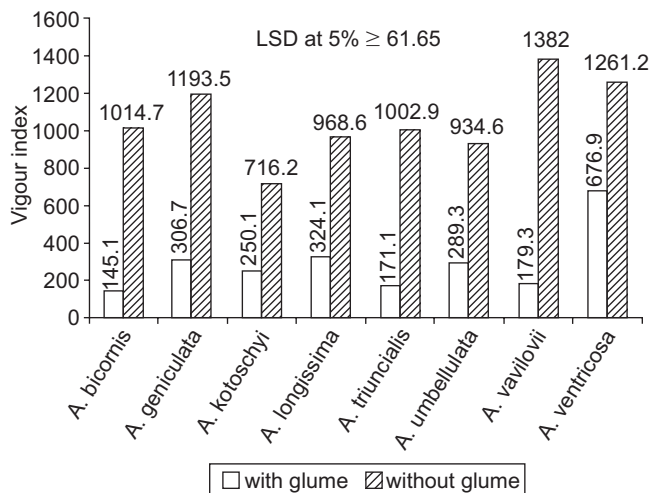


Fig 3 Effect of removal of glumes on the vigour index (VI) of *Aegilops* species

or mechanical restriction to embryo growth can be responsible for the low and delayed germination in the glumed seeds of *Aegilops* species.

Seedling vigour

In general, highly significant improvement in root, shoot and coleoptile vigour due to removal of glumes was recorded in all the *Aegilops* species investigated (Table 1). However, maximum improvement in root and shoot vigour (up to 2 or 3 fold) was observed in *Ae. longissima* and *Ae. triuncialis*. Highest improvement in coleoptile vigour was observed in *Ae. umbellulata* due to degluming of seeds. The boldest seeds were observed in *Ae. kotschyi* while smallest in *Ae. bicornis*. It is important to note that considering all the species tested, the trend in vigour index improvement in

Table 1 Effect of removal of glumes on root, shoot and coleoptile vigour of *Aegilops* species

Species	Root vigour (cm)		Shoot vigour (cm)		Coleoptile vigour (cm)		1000 seed wt (g)	
	With glume	Without glume	With glume	Without glume	With glume	Without glume	With glume	Without glume
<i>Ae. bicornis</i>	4.14	7.03	1.54	3.11	1.43	2.82	3.69±0.16	3.05 ±0.21
<i>Ae. geniculata</i>	7.81	8.50	2.42	4.06	2.15	3.95	57.1±1.77	12.45± 0.77
<i>Ae. kotschyi</i>	2.56	5.60	1.41	2.83	0.76	2.69	57.5±1.27	30.86±0.95
<i>Ae. longissima</i>	2.81	7.25	2.10	6.2	1.50	3.54	31.3±1.12	7.56 ±0.16
<i>Ae. triuncialis</i>	3.06	6.96	1.01	3.60	0.52	1.25	40.2±1.98	12.76 ±0.2
<i>Ae. umbellulata</i>	3.82	6.47	1.97	3.92	0.70	3.02	52.6±0.28	12.05 ±0.75
<i>Ae. vavilovii</i>	5.65	7.89	3.31	5.93	1.85	3.29	54.2±1.98	11.57 ±0.63
<i>Ae. ventricosa</i>	6.93	9.37	2.98	3.77	1.53	3.55	42.2±1.41	10.14 ±0.05
CD (P=0.05)								
V	0.215	0.158	0.134					
T	0.108	0.079	0.067					
V × T	0.305	0.223	0.190					

deglumed seeds in various species, in general, was similar to that of germination. It indicates that degluming of seeds of tested species improved significantly the germination as well as vigour of seeds. Our results are in agreement with several reports wherein removal of glume or lemma and palea (covering of seeds) increased the germination in several species of Poaceae (Simpson 1990). Germination in annual canary grass increased from 1 to 67% (Matus-Cadiz and Huel 2005), jointed goat grass up to 96% (Fandrich and Mallory-Smith 2006), in *Stipa tenacissima* from 20 to 97% (Gasque and Garcia-Fayos 2003).

To overcome the physical dormancy, generally mechanical and chemical methods are in vogue. In our study, although removal of glumes is skills-and labour-intensive, but it is very effective to increase the germination in all the species tested. We recommend that dormancy in seeds of *Aegilops* species can be overcome by removal of glumes. The germination, rate of germination and vigour can also be increased by this method. The genebank curators can follow this method to assess the initial germination before conserving such valuable donor germplasm on long-term basis in genebanks. Also the seed technology laboratories will benefit by using this method for routine determination of germination of these species.

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