RESEARCH ARTICLE

UTILIZATION OF WINTER HABIT DONOR, *AEGILOPS TAUSCHII* BY VERNALIZATION AND PHOTOPERIOD MANAGEMENT

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Received-05.12.2018, Revised-26.12.2018

Abstract: Allelic diversity in the wild grass Aegilops tauschii is vastly greater than that in the D genome of common wheat. Numerous efforts have been made to harness this extensive and highly variable gene pool for wheat improvement. This follows two distinct approaches, first production of amphiploids, between *Triticum turgidum* and *Aegilops tauschii*, and second direct hybridization between *Aegilops tauschii* and *Triticum aestivum*; both approaches then involve backcrossing to *Triticum aestivum*. Long duration, winter habit and specific requirements for raising *Aegilops tauschii* often make it difficult for every breeder to utilize the resource in their breeding programme. We demonstrate an easy low cost protocol for raising *Aegilops tauschii*, three times a year to facilitate the hybridization programs.

Keywords: Growth chamber, Faster breeding, Hybridization, Low cost

INTRODUCTION

Wheat breeding requires constant input of variation to attain higher yield. Owing to its own narrow genetic base, its progenitor and non progenitor species can be tapped to enhance the available variation. Among these, *Aegilops tauschii* Coss. commonaly known as goat grass is a wild diploid wheat relative and contributes the D genome to wheat (Kihara, 1944; McFadden and Sears, 1946). Wheat lines derived from those crosses have since been used in breeding programs worldwide and have helped farmers to boost yields by up to 20 percent. Goat grass is known for being highly adaptable and disease tolerant, so the crosses endow wheat with similar qualities. Varieties from these crosses make up over 30 percent of international seed stores (Rasheed *et al*., 2018). The D genome of *Ae. tauschii* was brought into the allohexaploid genome of common wheat through interspecific crossing to tetraploid wheat and subsequent amphidiploidization about 8000 years ago (Matsuoka, 2011). In contrast to the narrow geographic distribution of the other progenitor species, *Ae. tauschii* extends over a wide geographic range from eastern Turkey to China.

The fact that diploid D genome progenitor possesses a higher genetic diversity compared to bread wheat cultivars and landraces (Reif *et al*., 2005) makes it an ideal target for tapping novel genetic variation. *Ae. tauschii* has been used to introgress specific traits that include diverse resistance genes (Olson *et al*., 2013; Mandeep *et al*., 2010; Leonova *et al*., 2007; Miranda *et al*., 2006; Ma *et al*., 1993; Eastwood *et al*. ,1994), bread-making quality (Li *et al*., 2012), pre-harvest sprouting tolerance (Gatford *et al*., 2002; Imtiaz *et al*., 2008), yield (Gororo *et al*., 2002) and also morphological characters (Watanabe *et al*., 2006) into breeding material and cultivars of bread wheat.

Since the mid-twentieth century efforts directed at creating *Ae tauschii* introgressions into wheat has come from two avenues. Firstly, the more common approach of artificial hexaploid wheat synthesis that is generated by crossing tetraploid wheat with *Ae tauschii* and then doubling the triploid chromosome set by colchicine treatment or spontaneous doubling arising from unreduced gamete formation. Numerous reports on synthetic hexaploids have been reviewed by Ogbonnaya *et al*., (2013). Secondly, the process of direct introgression which involves *Ae tauschii* crosses with bread wheat where *Ae. tauschii* is the female parent and hexaploid as the male parent. The F_1 is either subjected to chromosome doubling and later backcrossed or is directly backcrossed repeatedly to recover a stable bread wheat derivative (Gill and Raupp, 1987). In this appraoach recombinant chromosomes between the diploid and hexaploid D genomes are produced. Introgression approaches that occur via synthetic hexaploids are not limited to the D genome but also involve the A and B genomes. As an alternative approach, wheat chromosome substitution lines carrying different chromosomes of *Ae. tauschii* were used in generating a set of well-characterized *Trtitcum aestivum/Ae. tauschii* introgression lines (Pestsova *et al*., 2006; Law and Worland, 1973).

MATERIALS AND METHODS

The production of synthetic wheat require hybridization which is simple and follows emasculation of durum wheat as female parent and *Ae. tauschii* is used as male parent for pollinations. Repeat pollination can be done or multiple spikes of tauschii can be used to ensure fertilization and seed set. Staggered planting or atleast three sowings of

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durums at regular intervals may be done, so that their flowering coincides with the flowering of the *Ae. tauschii* accessions. The F_1 seed set on durum wheat is observed and recorded. The F_1s are planted, these can be given chromosome doubling treatment to form stable synthetic wheat. Spontaneous doubling also occurs to some extent and can give stable synthetic hexaploid wheat.

In direct cross technique, the tauschii is taken as female parent and hexaploid wheat as male parent, post pollination support of auxin hormome like 2,4-D is given at 125 ppm concentration either as spray or drops to florets. In this cross, endosperm dose not form and developing hybrid embryo needs to be rescued and cultured over artificial medium. Post regeneration, the developing seedlings are hardened and given chromosome doubling treatment. On success of chromosome doubling, formation of octaploid will occur and these are fertile and can be utilized in backcross programme with hexaploid wheat, else the haploid ABDD can be backcrossed with hexaploid and some seed can be obtained which is again backcrossed for restoration of complete fertility.

RESULTS AND DISCUSSION

Direct crossing of *Ae. tauschii* with bread wheat is said to be the most ideal, efficient technique for exploiting Ae. tauschii variability for bread wheat improvement as this methodology rapidly produces improved BC_1. Alonso and Kimber., (1984); Cox *et al*., (1990,1991) and Gill and Raupp, (1987) unequivocally placed priority on crossing *Ae. tauschii* directly with bread wheat cultivars. Based on the transfer of stem rust resistance from *Ae. tauschii* to the bread wheat cultivar, Chinese Spring, Alonso and Kimber, (1984) determined that one backcross on to the F_l hybrids restored 92% of the genotype of the recurrent parent. In addition, the slight genotypic specificity seen in production of synthetic wheat, is ruled out here as any desirable wheat genotype can be used as the male parent e.g., 'Ciano T 79', 'Kanchan', 'Seri M 82','Opata M 85', 'Oasis F 86' and 'PBW 343' etc have been reportedly used for transfer of various traits (Mujeeb-Kazi *et al.*, 2006). Recently, the role of *Ae. tauschii* in drought and heat tolerance along with contribution towards yield components was established by work done at PAU (Chuneja, 2017; Arora *et al*., 2017). For transferring these traits, raising of tauschii was initiated and present study elaborates the protocol followed to raise the wild species multiple times a years to facilitate large number of crosses.

The basic requirement for conducting such crosses is raising of *Ae. tauschii*, a winter habit wild species along with durum wheat or bread wheat. Multiple sowing of durum and bread wheat is done so as to synchronize the flowering. *Ae. tauschii* when grown in normal conditions without vernalization, flowers when wheat season is almost over(Table 1). The number of ears and florets are less and often cleistogamous nature gets promoted owing to high temperature in the field. This renders it unsuitable for crossing; in addition wheat can be available only if raised in greenhouses and/or growth chambers. The synchronisation and timely flowering in *Ae. tauschii* can be instigated through specific measures. Vernalization treatment and photoperiod extension are two ways through which the flowering time manipulation can take place in *Ae. tauschii*. Most breeders utilizing tauschii provide it mostly extended photoperiod and go for staggered or multiple sowing of wheat or durum parents under controlled conditions. This provides single opportunity for conducting crosses. Though its an established fact that vernalization and photoperiod are basic requirement for winter habit genotypes, and should be given but no clear protocol is available for the same. We hereby present a standardized protocol for raising Ae. tauschii multiple times a year as per requirement. The partial controlled facility and largely field based protocol can be utilised by wheat groups working in Northern India.

Three distinct growing seasons have been identified, First, September to May, this is parallel to the main wheat season in north India and crossing can be undertaken during month of February and March. Second, growing tauschii at off season location Keylong (10500ft altitude, Himanchal Pradesh), (April to October) and flowering occurs in the month of July and third is early planting at Ludhiana where flowering occurs in November. All the three system require vernalization but extended photoperiod is not required in later two approaches. The vernalization treatment is given through simple domestic refrigerators for 6 weeks at 4^0C followed by fixation of the treatment at 15^0C in growth chambers for 12-15 days. The exposure to light is not made during 6 weeks of vernalization, as than we need to shift to growth chambers with lights and this leads to rising of temperature under light. Precise controlled conditions are required for temperature, in absence of which the treatment is not effective. During vernalization the seeds are frequently watered with ¼ MS solution. The moisture should be maintained but over watering and complete wet situation should be avoided. If some fungal infection is observed, use of carbendazim (wettable powder) is done to control the fungus. The treatment in simple refrigerators is effective and anyone can duplicate the same. The fixation is not required, when growing tauschii at offseason as field temperatures are already lower than required temperature at off season location. Further for utilizing the space and number of accessions specific measure like growing the seeds in petriplates (Fig 1 B) and germination paper bags

(Fig 1 C) as against small trays (Fig 1A) is recommended. The vernalization using petriplates also helps in easy transportation of the samples to other locations, in our case Off season at Keylong. Since the road passage is closed until May, the vernalization is initiated in April and 6 weeks later petriplates are carried to the off season location without damaging the vernalized seedlings.

Table 1. Comparison of *Ae. tauschii* growing periods in Ludhiana and Keylong

Duration	Time of flowering	Vernalization	Photoperiod	No of tillers	Suitability
September to May	Feb-March 170-180 days	6 weeks (Refrigerator) 2 weeks fixation (Growth chamber)	December-January	>50 6-8 florets	++
April to October	July 90-100 days	6 weeks (Refrigerator) 2 weeks fixation not required	Not required	30-50 More no. of florets (~12)	++++
July to February	November 120-130 days	6 weeks (Refrigerator) 2weeks fixation (growth chamber)	Not required	30-40 More number of florets (~12)	+
October-June (control)	April-May >190 days	Not given	Not given	Few tillers and florets	-

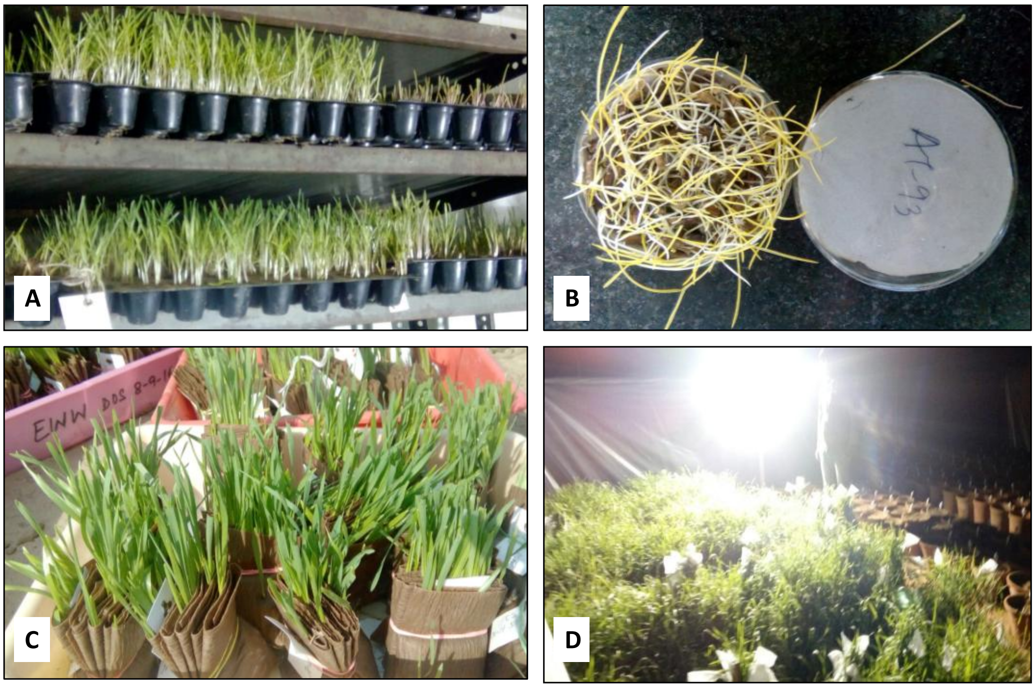

Figure 1: A) Vernalization and fixation of vernalization in plastic propagation tray B) Vernalization treatment in petriplates C) Vernalization treatment in germination paper pouches D) Extended photoperiod through use of halogen and/or LED lamps

Post vernalization and fixation, the seedlings are transferred to field. Pulverised soil bed is enriched with vermi-compost and standard dose of nitrogen, phosphorus and potash is provided. If possible soil application of zinc and sulphur can also be made for complete nutrition. Seedlings are regularly watered to promote establishment. The extension in photoperiod is provided through use of Halogen lamps (400 watt) and/or LED (50 watt) lamps (Fig 1 D) under filed conditions. The photoperiod is continued till flowering in initiated and once the accessions start showing the ears, the lamps can be removed. The tillering and ear size and floret number may vary (Table 1) but still is sufficient to conduct the crossing under any of the three situations against the control treatment. The comparison of three growing season shows that raising tauschii at off season location is best treatment as it requires less resources and time. Also the, flowering is quite determinate in nature over there, in addition it provides the next season in tandem to carry out chromosome doubling treatment. The protocol generated is low cost as it amalgamates field with controlled conditions. The vernalization treatment through domestic refrigerator makes the system applicable to institutes not have complete controlled facility. The only requirement of growth chamber is for 15 days duration between transition from refrigerator to field. Even this can be avoided or surpassed by choosing off season location to conduct the crosses. The three growing seasons provide flexibility in choosing the time as well as provides opportunity for corrective crosses.

In present, age when faster generation cycling protocols are coming up with six or more generation a year (Watson *et al* 2018), the concurrent use of tauschii under such situation was addressed by our study and it was established that three seed to seed cycles of *Ae. tauschii* can be grown in a calendar year.

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