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Hybrid Wheat: Development and Current Status

Introduction

International Food Policy Research Institute (IFPRI) projects that two-thirds of the world's wheat consumption will occur in developing countries and wheat imports are estimated to double by 2020 (Pingali, 1999). According to DWR, Karnal wheat production to feed the burgeoning Indian population will be around 109 million tonnes by 2025 AD (Mishra *et al.*, 2007). Agricultural constraints in developing countries, including India, like mounting population pressure, decreasing areas of cultivable land, changing climate and stagnating yield pose greater challenges for agricultural research to shift the crop yield frontier outward and into marginal areas that were often neglected in the past.

Hybrid wheat, a promising approach over open-pollinated varieties (OPVs) for improving yield potential, yield stability and pyramided stress tolerances by exploitation of heterosis, is attracting public and private-sector investment to address these challenges. Although heterosis is weaker in wheat, some hybrid wheat can increase yield by more than 15% when compared to the average of their parents, and even more in stressful conditions. A great effort went into hybridization during 1970's-1990's all over the world and much happened in USA, Australia and Europe. The results obtained were inconclusive in terms factor cost and the research was therefore abandoned. Cargill, which had been marketing hybrid wheat in the USA since 1981, finally gave up its research efforts in 1994. Fewer players exists today. But research into hybrid wheat has taken off again in asia, especially India and China.

History of hybrid wheat development

As on today Saaten-Union, a Germany based seed producer, is still the indisputable leader of hybrid wheat in Europe after purchasing major business stake from Monsanto ("Hybriditech" and its CHA "Genesis") and DuPont ("Hybridnova" and its CHA "Crolsor"). Saaten-Union alone produces 80% of world hybrid wheat requirement (*www.ble-hybride.com*). However, until now large-scale production of hybrid wheat has been

Hybrid wheat development: Timeline of major events

Year	Event
1934	Heterosis first reported in wheat for yield by Engledow and Pal
1951	Cytoplasmic Male Sterility introduced into wheat using *Aegilops caudata* cytoplasm (kihara)
1957-58	USA is the first country to plan and start CMS research on wheat in Kansas
1959	Nuclear Male Sterility reported in wheat by Pugsley and Oram
1961	Fertility restorer found in adapted wheat varieties "Gaines" is the first semi-dwarf wheat variety released in USA
1962	Source of CMS found in *Triticum timopheevi* by Wilson and Rose
1974	First commercial CMS based hybrid wheat released in USA
1984	OECD begins international certification scheme for hybrid wheat
1985	INRA's first hybrid "Courtel" is listed in the official French Catalogue. Commercial seed production failed due to inefficient CHA
1993	The CHA "Croisor" of Hybrid nova and "Genesis" of hybridtech, received a temperory permit to sell in France. Performance of CHA allows seed production economically viable
1995	DWR, an ICAR unit, initiated hybrid wheat programme in a network mode through CHA and CMS
2000-02	Saaten-Union take over of Monsanto Co. "Genesis" and DuPont "Croisor"
2003	DWR, Karnal and NCL, Pune got US patent (US 2003/0192070A1) for CHA inducing complete male sterility, its process of preparation and use
2003-05	Saaten-Union re-launches the sale of hybrid wheat in France and Germany
2006	Listing of HYMACH
2007	Listing of HYSTAR ICAR discontinued work on hybrid wheat through CHA
2009	ICAR initiated network project on hybrid wheat using CMS approach
2010	Listing of HYBERY, for miling quality Hybrid wheat occupies 1,70,000 ha in Europe with 1,40,000 ha only in France
2011	Listing of HYTECK, by Saaten-Union for biscuit making Listing of HYXTRA and HYXPRESS

prevented by the difficulty in developing reliable hybrid wheat seed production systems, with demonstrated yield advantages (Anonymous, 2011). With research efforts going into wheat across the globe, hybrid wheat programme in India was taken up by DWR, Karnal in a network mode employing use of CHA and CMS approaches. With all the research development, ICAR discontinued research on hybrid wheat development using CHA in 2007, but the CMS approach was still used. Private seed player Mahyco has released wheat hybrid PRATHAMA 7070, which now occupies around 40,000 ha in India

Problems in hybrid wheat breeding

1. Stability of CMS line, 2. Heterosis over best parent (Lack of genetic variability?), 3. Assembling favourable genes from diverse genetic resources, 4. Hybrid necrosis (Ne 1 & 2) and chlorosis (Ch 1 & 2), 5. Creation of heterotic gene pools, 6. Seed production of both A x B & A x R, 7. Constant release of average potential varieties & lines, 8. Low yield & high cost of seed and 9. Specific conditions of adaptations.

Having all these problems in hand, it seems hybrid wheat is a distant hope. Still scientists are over it. Two hybrid wheat varieties developed by the Beijing Academy of Agriculture and Forestry Sciences, including Beijing Wheat No6 and No7, have been successfully grown in pilot areas in Pakistan, and are expected to increase local wheat production by 50 per cent, said President of Farmers Association of Pakistan, Dr Tariq Bucha (2012). So, this is a high time to act efficiently.

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Mapping Seed Traits I: Mapping Populations and Molecular Markers

Seed quality is one of the most critical factor that effect the success of crops. High quality seed is collective attributes that contribute to the performance of seed under varied environmental conditions. However, interactions between these environemntal factors and seed's genetic make-up continues untill the death of seed. In crop species, huge natural phenotypic variations is recorded for the seed quality traits, paving way to identity genes controlling these traits. You list any seed quality related traits, they are ought to be complex and controlled by many genes and majorly influence the final yield. Mapping these traits increase the understanding of genetic basis and facilitate crop improvement via marker assisted selection.

Genetic mapping (also known as linkage or meiotic mapping) refers to the determination of the relative position and distances between markers along chromosomes based on the mean number of recombination events, involving a given chromatid, in that region per meiosis. Genetic map construction requires that the researcher develop appropriate mapping population, decide the sample size and type of molecular marker(s) for genotyping, genotype the mapping population with sufficient number of markers, and perform linkage analyses using statistical programs (Linkage1, GMendel, MapMaker, MapManager, JoinMap etc.). The construction of detailed genetic map with high level of genome coverage is the first step for localizing genes or quantitative trait loci (QTL) that are associated with economically important traits, marker assisted selection, comparative mapping between different species, a framework for anchoring physical maps, and the basis for positional cloning of genes. Hence, development of appropriate mapping population forms the basic platform for most of the molecular marker assisted plant breeding applications.

Mapping populations

A population used for gene mapping usually obtained from controlled crosses is commonly called a mapping population. The first and most critical step in producing a mapping population is selecting two genetically divergent parents, which show clear genetic differences for one or more traits of interest at phenotype level. Sufficient variation for the traits of interest at the DNA sequence is equally important to trace the recombination events for the easier identification of polymorphic informative makers.The parents should be genetically divergent enough to exhibit sufficient polymorphism and at the same time they should not be genetically too distant to cause sterility of the progenies and/or very high levels of segregation distortion during analysis. Consideration must be given to the source of parents used in developing mapping population. Genetic divergence between parents influences marker-trait associations and hence economic significance of map.

Development of mapping populations

Progenies from the second filial generation (F_2), F_2 derived F_3 (F_2:F_3) populations, backcross (BC) populations, recombinant inbred lines (RILs), doubled haploids (DHs), near isogenic lines (NILs), and immortalized F_2 population have been used for genetic mapping in crop plants. F_2 populations are developed by selfing F_1 hybrids derived by crossing the two parents while BC population is produced by crossing F_1 back into one of the parents (the recipient or recurrent parent)(Fig. 1). $F_{2:3}$ population is generated by selfing the individual F_2 segregants for a single generation. RILs are developed by single-seed selections from individual plants of an F_2 population; such selections continue for 6-8 generations. If backcross selection is repeated at least for six generations, more than 99% of the genome will be derived from recurrent parent. Selfing of selected individuals from BC_7F_1 will produce BC_7F_2 lines that are homozygous for the target gene, which is said to be nearly isogenic with the recipient parent (NILs). A DH population is produced by anther/pollen culture of F_1 plants. Plants will be regenerated using tissue culture techniques after induction of chromosome doubling from pollen grains or haploid embryos resulting from species crosses. Immortalized F_2 populations can be developed by paired crossing of the randomly chosen RILs derived from a cross in all possible combinations excluding reciprocals. The set of RILs used for crossing along with the F_1 produced, provide a true representation of all possible genotype combinations (including the heterozygotes) expected in the F_2 of the cross from which the RILs are derived. The RILs can be maintained by selfing and required quantity of F_1 seed can be produced at will by fresh hybridization. This population therefore provides an opportunity to map heterotic QTLs and interaction effects from multi-location data.

Characterization of mapping populations

Precise molecular and phenotypic characterization of mapping population is vital for success of any mapping project. Since the molecular genotype of any individual is independent of environment, it is not influenced by G × E interaction. However, trait phenotype could be influenced by the environment, particularly in case of quantitative characters. Therefore, it becomes important to precisely estimate the trait value by evaluating the genotypes in multi-locations over years using immortal mapping populations to have a valid marker-trait association.

Utilization of mapping populations

Currently available literature shows that genetic maps are

constructed using different types and sizes of mapping populations, marker systems, statistical procedures and computer packages. Each factor can affect the efficiency of the mapping process because of differences in the genetic distances between markers that can occur by variations in the degree of recombination observed in different crossings. Each mapping population has advantages and disadvantages and the research needs to decide the appropriate population for linkage mapping depending on project objective, time available for developing the population, and whether the molecular markers to be used for genotyping are dominant or co-dominant.

Both F_2 and BC populations are the best populations for preliminary mapping and are the simplest types of mapping populations as they are easy to construct and require only a short time to produce. However, F_2 and BC populations are ephemeral populations because they are highly heterozygous and seed derived from selfing these individuals will not breed true, so cannot be propagated indefinitely through seeds. This limitation can be overcome to a limited extent by cuttings, tissue culture or bulking F_3 plants to provide a constant supply of plant material for DNA isolation. Nevertheless, it is difficult or impossible to measure characters as part of quantitative trait locus (QTL) mapping in several locations or over several years with F_2 or backcross populations. Thus, the effect the G × E interaction on the expression of quantitative traits cannot be precisely estimated. The specific advantage of BC populations is they can be further utilized for marker-assisted backcross breeding. Both F_2 and BC populations are the products of one cycle of meiosis, so are of limited use for fine mapping. The $F_{2:3}$ population can be used for reconstituting the genotype of respective F_2 plants, if needed, by pooling the DNA from plants in the family. Though not an 'immortal' population, it is more suitable for mapping quantitative traits and recessive genes.

RILs, NILs and DHs are permanent populations because they are homozygous or 'true-breeding' lines that can be multiplied and reproduced without genetic change occurring. Repeated selfing of F_2 plants leads to RILs that each contains a different combination of linkage blocks from the original parents. The differing linkage blocks in each RIL provide a basis for linkage analysis. However, several generations of breeding are required to generate a set of RILs, so this process can be quite time-consuming. Moreover, some regions of the genome tend to stay heterozygous longer than expected from theory and obligate outcrossing species are much more difficult to map with RILs because of the difficulty in selfing plants. Nevertheless, in cases where it is feasible, seed from RILs is predominantly homogeneous and abundant, so the seed can be sent to any lab interested in adding markers to an existing linkage map previously constructed with the RILs. RILs being obtained after several cycles of meiosis are very useful in identifying tightly linked makers, thus making them ideal for QTL mapping.

Although NILs are frequently generated by plant breeders as they transfer major genes between varieties by backcross breeding, they are suitable populations for molecular tagging and functional genomics but not for linkage mapping. DH populations are completely homozygous inbred populations and are produced quickly than RILs and NILs but the production of DHs is only possible for species with a well established protocol for haploid tissue culture. Anther culture induced variability should be taken care of. So production of DHs demands relatively more technical skills and it accounts recombination only from male side compared to RILs and NILs.

Population size and marker systems

Once an appropriate mapping population has been chosen, the appropriate population size must be determined. The type and size of mapping populations can exert an influence on the accuracy and economic significance of genetic maps. Larger mapping population is always better especially when the goal is high resolution mapping in specific genomic regions or mapping QTLs of minor effect. Immortal populations of large size (preferably more than 200 individuals), genotyped by co-dominant markers yield more precise and high resolution linkage maps.

Different molecular marker systems *viz.*, restriction fragment length polymorphisms (RFLPs), microsatellites or simple sequence repeats (SSRs), expressed sequence tags (ESTs), cleaved amplified polymorphic sequence (CAPS), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs), inter simple sequence repeats (ISSR), diversity array technology (DArT), and single nucleotide polymorphism (SNP) have been used for map construction in several crops. Each marker system has its own advantages and disadvantages. For high throughput screening and high resolution mapping, molecular markers should preferably be highly informative, co-dominant, reproducible, locus specific, cross-transferable, and amenable to complete automation. Although, the scope of EST-derived marker development is limited to species for which sequencing databases already exist, but wherever possible, EST marker should be exploited for mapping as, it is found to be genetically associated with a trait of interest, so offers possibility of direct mapping of trait, candidate gene based and comparative mapping across different species.

Combining markers and populations

The genetic segregation ratio at maker locus is jointly determined by the nature of marker (dominant/ co-dominant) and types of mapping populations (Table 1). Therefore, a thorough understanding of the nature of markers and

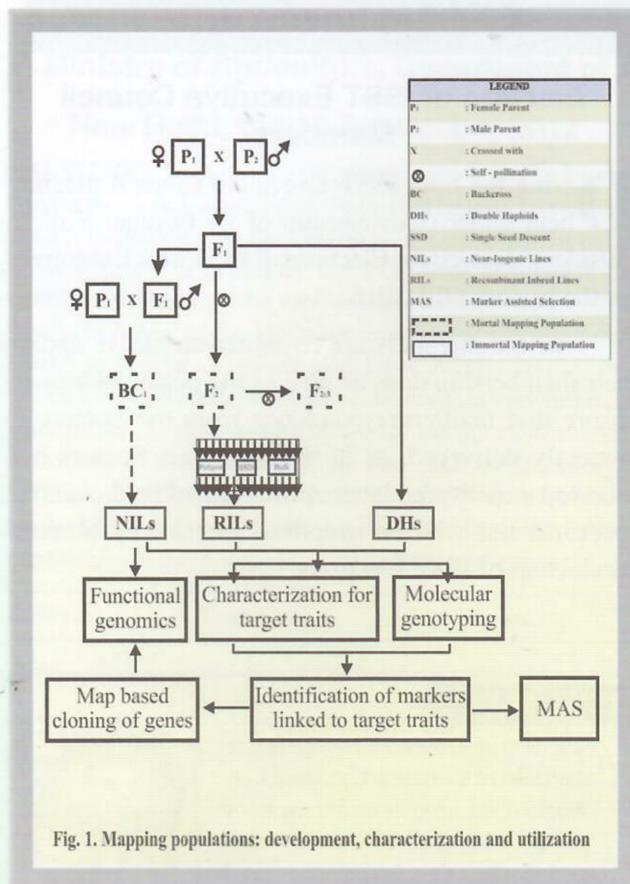

Fig. 1. Mapping populations: development, characterization and utilization

mapping population is crucial for any mapping projects. Markers such as RFLPs, microsatellites and CAPS etc. are codominant in nature, while AFLP, RAPD, ISSR are often scored as dominant markers. Mapping populations such as RILs and DHs equalize marker type because of fixation of parental alleles at marker locus in homozygous condition. These populations result in 1:1 segregation ratio at marker locus irrespective of genetic nature of markers, while an F_2 population segregates in 1: 2: 1 ratio for a codominant marker and in 3:1 ratio for dominant marker (Fig. 2). Depending upon the segregation pattern, statistical analysis of marker data will vary.

Maximum genetic information is obtained from F_2 population using a co-dominant marker system. Dominant markers supply as much information as co-dominant markers in RIL, NILs and DHs because all loci are homozygous, or nearly so. Information obtained from BC populations using either co-dominant or dominant markers is less than that obtained from F_2 populations because one, rather than two, recombinant gametes are sampled per plant. RILs, NILs and DHs may be powerful tools for QTL detection in some circumstances but provide no information on dominance relationships for any QTL while, F_2 is preferred for detecting QTLs of additive effect.

Table 1. Genetic segregation ratio at marker locus in different marker - population combinations

Mapping populations	Genetic segregation ratio for Codominant marker	Dominant marker
F_2	1:2:1	3:1
RILs	1:1	1:1
DHs	1:1	1:1
NILs	1:1	1:1
Backcross population		
B_1	1:1	1:0
B_2	1:1	1:1

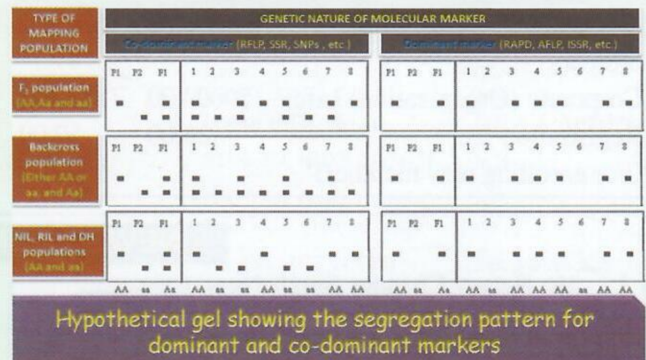

Fig. 2: Characterizationof mapping population vis-a-vis different marker system (adopted from Semagn *et al*., 2006)

Suggested Readings

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ANNOUNCEMENT FROM INDIAN SOCIETY OF SEED TECHNOLOGY, NEW DELHI

Revision of ISST Membership Fee

The 69th ISST Executive Committee Meeting held on September 15, 2012 approved the revision of membership fee *w.e.f.* April 1, 2012 taking into cognigence of prevailing membership from April 1, 2006.

Kind of Membership	Existing Fee (Rs.)	Revised Fee (Rs.)
Ordinary (individual)annual	200.00	500.00
Life (individual)	2400.00	6000.00
Associate (Organization) ordinary	4000.00	6000.00
Corporate (Organization) Life	50000.00	75000.00
Registration fee (For enrolling new member)	25.00	50.00

Election of ISST Executive Council Members

During the 69th ISST Executive Council meeting held under chairmanship of Dr Gautam Kalloo, President, decided for Elections of ISST new Executives for the period 2013-2016.

All the members are requested to please update their membership dues as well as the postal address to ensure that mail/correspondence from the Society is correctly delivered, as in the near past Society has received undelivered letters/posts. Based on the above, electoral list will be prepared to take systematic conduction of ISST Elections.

NEWS FROM DSST, IARI

1. Sh. Harish Chander Singh Negi, Senior Technical Officer, superannuated from his official duties on September 30, 2012. A kind, social and humble human, Sh. Negi has spent his career in Seed Testing services at Division of Seed Science and Technology and volunteered in perfectly managing all activities of the Division. He has contributed for the cause and development of ISST and is presently its Treasurer. Amongst collegues and friends he is popular for his positive helping attitude, wisdom and loyalty. ISST wishes for a fruitful retirement life and look forward for his continuous support.

2. Dr. Virender Singh Lather, Principal Scientist has joined the team of scientists at Division of Seed Science & Technology, IARI on 18 December 2012. Born on April 5, 1955, he earned his Ph.D. in Genetics and Plant Breeding from GPUA&T, Pantnagar. He has 33 years of teaching and research experience in the field of Genetics/Cytogenetics. Worked on crop improvement of pulses (chickpea and mungbean) and oilseeds (rapeseed and mustard). ISST welcomes him to the Seed Science faternity and wishes him a fruitful career at DSST, IARI.

3. Division of Seed Science & Technology and Division of Genetics had jointly demonstrated technologies on quick tests for DNA fingerprinting (for genetic purity), TZ test (for seed viability) and Phenol test (for wheat varietal identification) at Agricultural Pavilion put up by Ministry of Agriculture, Govt. of India during India International Trade Fair, 2012 from November 14-27, 2012. The Agricultural Pavilion attracted large number of farmers and visitors.

Editorial Contact Information

Please send us information related to any news, new projects, opinions on policy issues, current happenings, publications, book reviews, foreign visits, new appointments, trainings, seminars, workshops and conferences or other interesting stuff related to seed for the next issue of Seed Tech News.

Suggestions and comments are welcome!

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NOTIFICATION

Ministry of Agriculture, Department of Agriculture and Co-operation, Govt. of India

New Delhi, the 10 September 2012

S.O. 2125(E) - In exercise of the powers conferred by Section 5 of the Seeds Act, 1966 (54 of 1966), the Central Government, after consultation with the Central Seed Committee, is of the opinion that it is necessary and expedient to regulate the quality of the seeds of the varieties specified in column (2) of the Table below of the kinds specified in the corresponding entries in column (1) of the said Table, hereby notifies that the said varieties of seeds shall be the notified varieties to be sold for the purpose of agriculture for the States mentioned in column (3) of the said Table and shall be the notified varieties for the whole of India for the purpose of seed production and quality control with effect from the date of publication of this notification in the Official Gazette.

Kind (1)	Variety (2)	States (3)
Barley	BH 885	HR
Brown Sarson	Shalimar Sarson-1	JK
Castor (Hybrid)	DSP 222	GJ, RJ, UP & HR
Chickpea	PKV Harita (AKG 9303-12)	MH
Cotton	Phule Anmol (RAC 024)	MH
	Phule Dhanwantary (Rh. arb. 02-1)	MH
Fingermillet	Indira Ragi-1 (BR-7)	CG
Grain Amaranthus	Phule Kartiki (RGAS 92-10-1)	MH
Kodomillet	Indira Kodo-1 (BK-1)	CG
Maize (Hybrid)	KMH-218 Plus	UP, BR, OR&JH
	KMH-3426	UP, BR, OR, JH, RJ, GJ, MP & CG
	NMH-731	GJ, RJ, MP&CG
	NMH-803	UP, BR, JH, OR, GJ, RJ, MP & CG
	HM-12 (HKH 313)	UP, BR, JH&OR
	KMH-25K60	AP,KA,TN&MH
	KMH 3712	PB, HR, DL, UP, BR, OR, JH, RJ, GJ, MP & CG
	NMH-920	UP, BR, JH&OR
	Bisco x 1 (Bisco 506)	UP, BR, AS, WB, OR, AP, KA, MH & TN
	P3441 (X8B691)	UP, PB, HR, BR, OR,JH,MP,GJ&RJ
	P3502 (X8B562)	MP, RJ & GJ
	NK-30 (NECH-132)	PB, HR, DL, UP, BR, JH, OR, KA, AP, TN, MH, RJ, GJ, CG & MP
	NK 6240 (NECH-131)	PB, HR, DL, UP, KA, AP, TN & MH
	BIO-9682	MP, RJ, GJ, UP, PB & HR
Mungbean	BM 2003-2	MH
Pearlmillet	PKV-Raj (BBH-3)	MH
	ABPC-4-3 (MP 484)	MH
Pearlmillet (Hybrid)	Kaveri Super Boss (MH 1553)	RJ, GJ, HR, UP, PB, MP, MH, KA, AP & TN
	Bio 70 (MH 1632)	RJ, GJ & HR
	Bio 448 (MH 1671)	RJ, GJ, HR, MP, UP, PB &DL
	Nandi-70 (MSH 224) (NMH 73)	GJ, RJ, MH & TN
	Pratap (MH 1642)	MH,KA,AP&TN
Pigeonpea	BDN 711 (BDN 2004-3)	MH
Rice	JGL 3844 (Jagtial Samba)	AP
	JGL 3828 (Manair Sona)	AP
Rice (Hybrid)	US 382 (IET 20727)	TR, MP & KA
	Arize Tej (HRI 169) (IET 21411)	BR, CG, GJ, AP & TN
	PNPH 24 (IET 21406)	BR, WB & OR
	NPH 924-1 (IET21255)	WB & AS
	NK 5251 (IET 19738)	TN, KA, AP, MH & GJ
	27P31 (IET 21415)	JH,MH, KA&TN
	27P61 (IET 21447)	CG, GJ, AP & KA
	25P25 (IET 21401)	UK, JH & KA
Safflower	SSF-708	MH
Sesamum	JLT-408 (JLS-9848-2)	MH
Sorghum	CSV 26 (SPV-1829)	Entire country
	Phule Panchami (RPOSV3)	MH
	Phule Revati (RSV 1006)	MH
Sugarcane	Co 0237	PB, HR, RJ, UK & UP
	Co 0403	TN,AP,KA&MH
Triticale	TL 2969	JK, HP, UK, SK, WB & NESs
Wheat	PDKV Washim (WSM 1472)	MH

S/d-

Atanu Purakayastha, Jt. Secy.

[F. No. 3-16/2012-SD.IV]

AP: Andhra Pradesh, AS: Assam, BR: Bihar, CG: Chhattisgarh, DL: Delhi, GJ: Gujarat, HR: Haryana, HP: Himachal Pradesh, JK: Jammu & Kashmir, JH: Jharkhand, KA: Karnataka, MP: Madhya Pradesh, MH: Maharashtra, NESs: North Eastern States, OR: Orissa, PB: Punjab, RJ: Rajasthan, SK: Sikkim, TN: Tamil Nadu, TR: Tripura, UK: Uttarakhand, UP: Uttar Pradesh, WB: West Bengal.

M.Sc. Thesis Abstracts

Studies on Pollination Ecology, Seed Development and Seed Quality Enhancement in Black Cumin

Black cumin (*Nigella sativa* L.) is an important seed spice of India and its seed oil has important application in pharmaceutical industry. Pollination timing, seed development & maturity and seed quality enhancement needs special attention in this crop. Experiment on pollination ecology at DSST, IARI showed that *Nigella* was able to set seeds under open, selfing and cross pollinated condition and no significant difference in seed yield was observed among the different pollination conditions. The maximum pollinator's activity was observed from 10.30 am to 11.30 am during 3rd week of Feb. 2012. *Apis florea fabricius* was the most common among pollinators followed by *Apis cerana cerana fabricius*. Studies on seed development and maturation showed that seeds reaches to harvest maturity at about 45 days after pollination (DAP) while onset of germination takes place at about 35-40 DAP. Acquisition of desiccation tolerance was obtained after 40-45 DAP when induction of dormancy took place. Seed enhancement studies in three different seed lots showed that, priming enhanced germination and vigour in Lot 2 with low initial germination (80%), while no significant enhancement in germination and vigour was observed in Lot 1 and 3 with high initial germination (94% and 93%, respectively). There was decline in germination and vigour of all three primed seed lots after three months of ambient storage indicating that priming of seeds do not maintain seed viability and vigour during storage.

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Application of Molecular Markers for Testing the Genetic Purity of Pearl Millet Parental Lines and Hybrids

Conventionally the genetic purity of the hybrids is assessed by grow-out-test (GOT). However, molecular marker being more reliable and reproducible can be employed to overcome many limitations of GOT. The present study was undertaken to identify the SSR markers distinguishing eleven pearl millet hybrids and their respective parental lines. Among the 40 SSR markers studied, 9 markers (*viz.*, PSMP2084, PSMP2203, PSMP2040, PSMP2089, PSMP2202, PSMP2237, PSMP2273, PSMP2270 and PSMP2263) were found to be suitable for testing the genetic purity of ten respective hybrids namely, RHRBH8609, RHRBH8924, GHB538, GHB 732, GHB 744, GHB 719, GHB 558, PUSA 605, PUSA 23 and HHB 67 improved. Cluster analysis based on Jaccard's Similarity Co-efficient using UPGMA grouped the hybrid into four major clusters. Within the cluster all the hybrids shared a female (A-Line) parent that was related to each other in their lineage. The genetic similarity between the hybrids ranged from 0.68 to 0.94 with an average similarity index of 0.79. The polymorphic markers so identified can be used as referral markers for unambiguous identification and protection of these hybrids. The analysis of plant to plant variation within the parental lines of all the hybrids, using the identified hybrid specific markers, showed highly homogenous profile of SSR markers, with greater scope of application in maintenance and seed purity testing of hybrids and parental lines. Genetic purity analysis of commercial seed lot of hybrids RHRBH 8609 and HHB 67 showed 3-4 per cent maintainer (B) line admixture in hybrid seed. This is the first report which demonstrates application of SSR markers for testing genetic purity of commercial seed of pearl millet hybrid.

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