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Enhancement of Genetic Variation through Different Appraoches in Wheat Breeding

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ABSTRACT

Wheat is self-pollinated crop and its evolution from wild grasses lead to its successful crossing with Aegilops, Agropyron and other species like rye allows adding new variations making it more diverse genetically. With a high selection pressure for desirable agronomic traits while attempting elite x elite crosses, diversity in the available germplasm is shrinking slowly. There is need to initiate the pre-breeding activity for developing genetic stocks for yield component lines coupled with resistance to biotic stress. Introgression of new genetic diversity from unrelated wheat germplasm can create new genetic pool and bring quantitative genes that may not be present in wheat germplasm commonly used in a breeding programme. Breeding wheat varieties with increased grain yield potential, enhanced water-use efficiency, heat tolerance, end-use quality, and durable resistance to important diseases and pests can contribute to meeting atleast half of the desired production increase and remaining half must come through better agronomic management practices.

Key words: *Breeding approaches, Genetic variation, Prebreeding, Wild relatives, Wheat*

INTRODUCTION

India is one of the major wheat growing and consuming countries of world and is second largest producer of wheat, after china. The cultivation of semi-dwarf wheat varieties made a significant contribution towards green revolution and as a result, India has achieved remarkable increase in production and productivity of wheat since mid-sixties, wheat production in India increased more than seven fold after 1965 (from 12.3 million tones in 1965 to 93.50 million tons in 2015-16) Figure 1. The genetic gains in yield potential of irrigated wheat stand at around 1% annually through varietal improvement efforts, while world demands for wheat is growing at approximately 2% per year. Thus global demand for wheat is growing at about twice the current rate of gain in genetic yield potential, with progress in rainfed environments being even lower[13].

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There is need to follow some new approaches in wheat breeding for making major gain in yield potential in breeding in order to develop new genotypes that are responsive to high input management and capable of yielding beyond 7 t/ha, to meet the future wheat requirement. Sustainable wheat production with reduced inputs of agrochemicals and developing lines and enhanced quality for specific end-uses and food security worldwide are the major concern for present as well as future[12].

Diverse Agroclimatic Zones of Wheat in India:

Wheat crop in India is grown under six diverse agro-climatic zones[1] *viz*., Northern Hills Zone (0.8 m ha) (Hilly parts of Jammu and Kashmir, Himachal Pradesh and Uttrakhand), North Western Plains Zone (11.3 m ha) (Punjab, Haryana, Western Uttar Pradesh and some parts of Rajasthan), North Eastern Plains Zone (9.5 m ha) (Eastern Uttar Pradesh, Bihar, Jharkhand, West Bengal, Orissa and Eastern States), Central Zone (5.2 m ha) (Madhya Pradesh, Chhatisgarh, Gujarat, Southern Rajasthan and Bundellkhand region of Uttar Pradesh), Penninsular Zone (0.1 m ha) (Maharashtra, Andhra Pradesh, Karnataka and Plains of Tamil Nadu) and Southern Hills Zone (0.1 m ha) (the Nilgiris and Palni Hills of Southern Plateau). This classification has been based on climatic conditions, soil types and growing duration of wheat. The crop however is grown across the country but nearly 70% lies in the Northern Plains and 20% in Central India. The Indo-Gangetic plains comprising the North Western Plains Zone (NWPZ) and the North Eastern Plains Zone (NEPZ) from the major wheat tract followed by the Central Zone (CZ) and the Peninsular Zone (PZ). Although wheat production has increased considerably during last several decades, yet the average yield continues to be low and regional disparity exists. The yield gaps between potential (best yield at research station) and realizable (yield harvested by farmers) vary from one zone to another zone. As per the estimates, maximum yield gaps exist in NEPZ followed by CZ, PZ and NWPZ. The two most important zones which account for about 80 per cent of the total wheat area in India are NWPZ and NEPZ and therefore, the wheat production in the country is mainly dictated by the wheat production in these two zones.

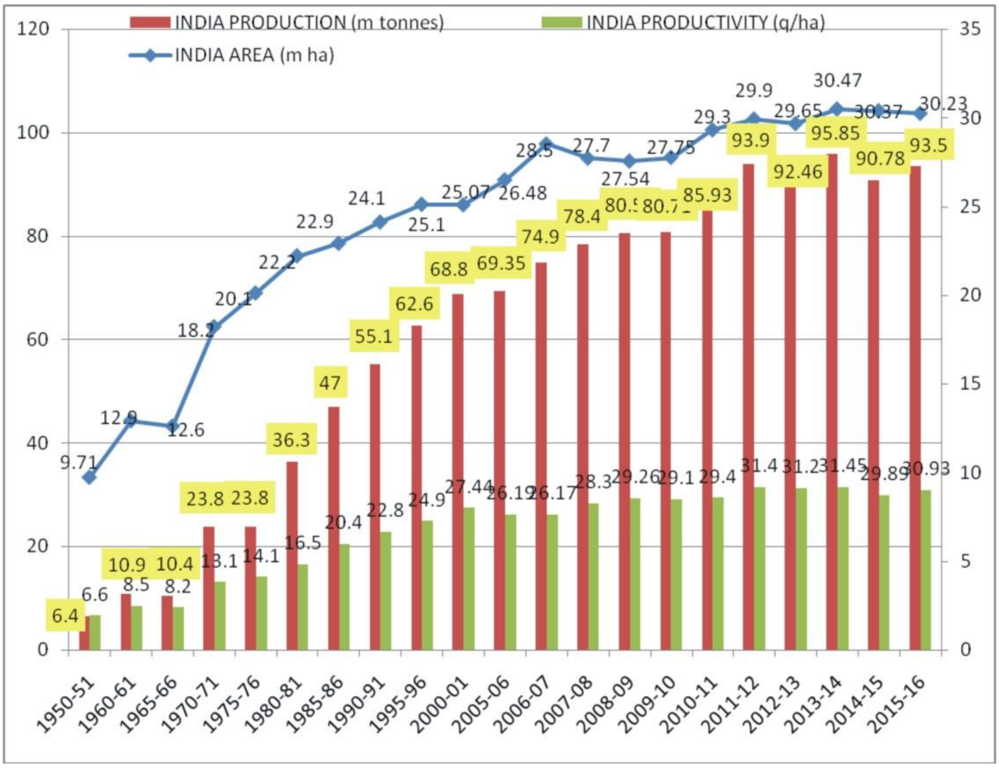

Fig. 1: Wheat area, production and productivity trend in India (1950-2016)

Expansion of Genetic variation

One of the main requirements of a dynamic programme is the easy access to the diversity for yield contributing genes and resistance to biotic and abiotic stresses. With a high selection pressure for few agronomic traits while attempting elite x elite crosses, diversity in the available germplasm is shrinking slowly. To augment working germplasm, there is need to initiate the pre-breeding activity for developing genetic stocks for yield component lines coupled with resistance to biotic stress. Introgression of new genetic diversity from unrelated wheat germplasm can create new genetic pool and bring quantitative genes that may not be present in wheat germplasm commonly used in a breeding programme.

Utilizing Buitre and Synthetics

For creating new genetic variability, emphasis has been on least exploited germplasm like long spike buiture plant types, synthetic hexaploids, Chinese sub compactoid ear head material and wild relatives. Long spike Buitre germpalsm developed by Ricardo Rodriguez at CIMMYT, Mexico has a robust stem, a long spike, more spikelets and more grains per spike, large leaf area and broad leaves but lack in grain traits, resistance to rust diseases, late maturity, shy tillering and stability in performance. A large number of synthetic hexaploids developed at CIMMYT, Mexico[5] with genetic richness for high grain weight, delayed senescence (stay green), HMW glutenins, resistance to KB and yellow rust are also in use. However, this germplasm had certain inherent limitations such as tall stature of plant, hard threshing, red grain colour, delayed heading and maturity associated with synthetic hexaploids.More than 25% of all new wheat that CIMMYT has distributed in recent past in nurseries has been synthetic derivatives. China has crossed CIMMYT synthetic wheat with local wheat and derived two varieties having 20 to 35% higher yields. One of these varieties Chuanimai-42 had yields of 6 tons/ha in trials and released in Si Chuan in 2003. Spain also registered synthetic wheat derivatives under name Carmona in 2003. It is fast growing high yielding short duration variety with better grain quality, disease resistance and suited to zero tillage. This synthetic hexaploid germplasm can also be exploited for resistance to leaf, stripe rust, drought, salinity, mineral toxicity[9] and heat, cold, sprouting, water logging[19], powdery mildew, loose smut, cereal cyst nematode (CCN) and better for yield components.

Alien Gene Transfer

An alien gene transfer in wheat means when one or a group of genes is transferred to wheat from some other species. This transfer of genes, chromosome segments or of whole chromosomes may be from a closely related or distantly species. The success of transferring alien genetic material to wheat is generally determined by the degree of pairing between the chromosomes of alien species and those of wheat. However, by producing amphidiploids (hybridization between two species followed by doubling of chromosomes), we not only can add or substitute whole chromosome or chromosome pair (production of addition and substitution lines), but also the whole genome of a species can be transferred to wheat (production of new species). Triticale or Triticosecale is the only one example of such man-made crop species, where whole genome of rye (*Secale cereale*) has been transferred to wheat. Nevertheless, rye has some undesirable genes too which have come in triticale along with its desirable characteristics. This is the biggest disadvantage of whole genome transfers and this is also biggest reason that triticale till today could not take the form of a completely successful crop species. Of course, in some countries of the world, triticale is being grown on commercial scale. *T. tauschii* (annual wild relative of wheat, an unequivocally accepted D genome donor to bread wheat) has a wide range of useful characteristics such as resistance to biotic and abiotic stresses, potent source of new variability for grain weight, bread making quality and photosynthetic efficiency. Since D genome of *T. tauschii* is also present in *T. aestivum*, the crosses between bread wheat and *T. tauschii* are considered as interspecific and not intergeneric by the CIMMYT scientists.Synthetic wheats, developed by crossing *Triticum turgidum durum* varieties as female and desired *T. tauschii* accessions as male parent followed by embryo rescue and chromosome doubling are being used widely in CIMMYT wheat improvement programme to enhance and introduce new genes especially for stress tolerance. Two wheat cultivars with synthetic wheats as parents are now released in China and Spain. An important trait derived from synthetic wheat is the better root characteristics that are probably associated with enhanced drought tolerance of synthetic wheat derivatives[14].

Several reports of transfers involving small chromosome segments or single genes for disease resistance in wheat from its related species or genera are available in the literature. Friebe *et al*[3] gave a long list of wheat-alien translocations. Some of these translocations obtained through homeologus recombination (e.g., T2B/2G#1 carrying Sr36/Pm6 genes from *Triticum timopheevii*), through irradiation (e.g. 7DS.7DL-7Ae#1L) carrying Lr19/Sr25 genes from *Agropyron elongatum* and 6AS.6AL-6Ae#1L carrying Sr26 from *A. elongatum*) or through spontaneous translocation (e.g. T3DS. 3DL-3Ae#1L carrying Sr 24/Lr24 genes from *A. elongatum* and T1BL.1R#1S (Wheat-rye translocation) carrying Lr 26/Sr31/Yr9/Pm8 genes from *Secale cereale*), have made significant contribution towards development of new wheat varieties and some of these genes are still effective is wheat growing belt of India. Nevertheless, the 1B/1R translocation (a simple transfer between rye and wheat in the Soviet Union cultivar KavKaz) carries a number of useful genes from rye and has contributed towards high yield, multiple disease resistance and adaptation of marginal environments. Crossing of spring wheat germplasm with winter wheat germplasm in the 1970s and 1980s not only increased the biomass of spring wheats but it also increased yield potential as observed in numerous cultivars released in many countries from **‘Veery’** and **‘Attila’** crosses of CIMMYT. Although thousands of crosses were made in this effort, only the above two crosses with winter wheat parents ‘Kavkaz’ and ‘Nord Desperez’, respectively can be considered most successful as they led to development of mega-cultivars released in East African highlands, North Africa, Middle East, Asia and Americas and subsequently grown on millions of hectares and are present in more than 250 cultivars world over. These cultivars showed 8-10% higher yield potential over previously developed cultivars. Alien translocation T7DS.7DL-7Ae#1L from *Agropyron elongatum* that carries leaf and stem rust resistance genes Lr19 and Sr25, respectively, has been shown to increase yield potential ranging from almost non-significant levels to over 15% depending on genetic background under irrigated conditions through increased biomass production caused by increased photosynthetic rate. Thus its widespread incorporation could lead to a quantum jump in yield potential. The initial translocation carried a gene that caused higher endosperm pigmentation that is an undesirable quality trait for bread wheat. However, Knott[4] developed a non-yellow mutant and this non-yellow variant translocation maintains the yield enhancing effect.Famous leaf rust gene Lr19 was derived from Agropyron by Canadian Scientist Dr DR Knott and later made available to CIMMYT through Purdue University. CIMMYT scientist Ricardo Rodgriguez, a pre-breeder, successfully transferred gene into a Bluebird 2 background in mid 1980. Later, Mexican NARS released wheat cultivar Oasis 89 carrying Lr 19. Cultivar is double dwarf like parent Blue bird 2 (Yecora 70) but less acceptable to farmers in Mexico due to quality issue[10].

Breeding methods:

Shuttle breeding method

One of the first things Dr. N.E. Borlaug realized when he initiated breeding in Mexico in the 1940s was the long time required to breed new cultivars if he continued with one generation per year. This prompted him growing two crop seasons per year at two sites in Mexico, Ciudad Obregon (28ºN latitude, 39 masl altitude) and Toluca (18ºN latitude, 2640 masl altitude) that have very distinct photoperiods, temperature and rainfalls during the crop seasons as well as distinct diseases. This “Shuttle Breeding” not only reduced the breeding cycle time by half but also allowed development of widely adapted wheat germplasm. This method is a combination of pedigree method and modified bulk method. The main objective of the CIMMYT scientist to use this method are to combine input efficiency and input responsiveness of wheat germplasm using alternate sites of contrasting environmental conditions and to breed wheat for drought tolerance for semi-arid regions of the world. As regards shuttle breeding programme in Mexico, segregating wheat populations are shuttled between the two environmentally contrasting locations Ciudad Obregon (dry, sunny, fertile and irrigated site situated at sea level in North West Mexico) and Toluca (a cool high land environment with high humidity, situated near Mexico city). The yield potential of Ciudad Obregon is high (about 10 tonnes/ha) and only few wheat diseases (leaf rust, Karnal bunt and black point are prevalent in the area. At this location, planting of wheat is done in November (low temperature period) and harvesting is done in April/May (high temperature period). On the contrary, at Toluca location, planting is done in May/June and the material is harvested in October. Since this location is characterized by high humidity, there is incidence of many wheat diseases. Shuttle breeding continues to be a very powerful tool to select for traits with simple or complex inheritance at relatively low cost. CIMMYT has extended shuttle breeding between Mexico and Kenya to enable selection of complex durable adult plant resistance to Ug 99 race of stem rust pathogen that threatens wheat production globally. This strategy is probably the best option at present to enhance heat tolerance in wheat materials adapted to North Western India[15]. Hence, different methods of breeding in wheat should be considered supplementary rather than antagonistic to each other and thus a method combining merits of two or more method will be more effective than a single traditional method.

New breeding methodologies

Method of breeding has also changed over years. Wheat breeding at CIMMYT in the 1960s and 1970s relied on simple, top and double crosses followed by pedigree method of selection. It was realized later that best advanced breeding lines were rarely derived from double crosses possibly because the genetic variation generated by such crosses was too large and the chances of recovering plants with desirable combinations of genes were reduced due to insufficient population sizes. During early 1980s, CIMMYT breeders relied upon simple and three-way crosses and occasionally single backcrosses followed by modified bulk selection[11]. Following the study by Singh *et al.*[16] which showed that selection schemes had little or no effect on the performance of progeny lines but the choice of parents determined the progeny response, a selected bulk breeding scheme was introduced in bread wheat at improvement during mid. 1990s. In all segregating generations until F_5 or F_6, one spike from each of the selected plants is harvested as bulk and a sample of seed is used in growing the next generation. Individual plants or spikes are harvested in the F_5 or F_6 generation. This scheme allows retaining a larger sample of selected plants without increasing the cost. Moreover, retaining a large sample of plants in segregating populations increases the probability of identifying rare segregates that carry most desired genes. Selected spike bulk has also been found effective in handling large population with limited resources[2, 17].

Modified selection and crossing scheme

A single backcross crossing approach that was initially applied to incorporate resistance to rust diseases based on multiple additive, minor genes, was found to favor selection of genotypes with higher yield potential as it favors retaining most of the desired additive genes from the backcross or recurrent parent while simultaneously allowing the incorporation and selection of additional useful small effect genes from the donor parent. The shift in mean results in higher frequency of new breeding lines that have superior yield potential than the check cultivar. Repeated backcrossing is not desirable as it was devised to incorporate a single or a few, major gene(s) with least disturbance to the genetic make-up of the recurrent parent[14]. Selective intermating has also been found to be effective for increasing the mean yield and its components in wheat[6,8,7]. Further, recycling of F_5 and F_6 materials as parents in crosses can also be included and in some cases the intercrossing of F_1's can also increase appearance of new recombinants.Keeping in view the importance of genetically diverse material in varietal development as evident from origin of semi-dwarf wheat (Figure 2), CIMMYT has maintained a wide genetic base in its breeding materials and continues to expand it (Table 1). The first successful cross II-8156 can be traced to 35 landraces. Over the years, this number has constantly increased to minimize the risk of genetic vulnerability since it is grown on large area and is widely used in developing countries. The use of genetically diverse material in crossing programme will be desirable to break undesirable linkages and increase the frequency of desirable alleles for future increase of yield potential and stability. The best performing lines often had genotypic base of widely adapted genotypes i.e. Kauz, Attila, Baviacora and Pastor with genetic contributions from other parents including synthetic wheat. Use of germplasm like McFadden's Hope

(Sr2 gene complex) for durable stem rust resistance and Frontana (Lr 34 gene complex) for durable leaf rust resistance has also reduced stem and leaf rust vulnerability, respectively.

Table 1: Analysis of percentage of four important CIMMYT wheat crosses (George Varughese[18])

Year	Cross name	Number of land race parents in background
1966	II-8156	35
1977	Veery	48
1985	Kauz	51
1990	Attila	63

Use of Mutation Breeding

Despite the fact that mutation breeding has some important advantages (The technique excels other) breeding procedures, in its potential for creating new genes and gene combinations in a species; the technique facilitates induction of any kind of heritable change right from point mutation to the loss or addition of entire genome; and new advancements and refinements in this technique allow induction new genes at specific loci), the technique has some serious drawbacks too. The main drawbacks , of mutation breeding technique are: (i) The proportion of desirable mutations in the total number of mutations produced is very low as most of the mutations are recessive and harmful; (ii) the success of this technique greatly depends on the availability of a suitable screening technique; (iii) in some cases, association of harmful side effects with useful mutations, makes the job of the plant breeder more difficult; (iv) the technique is relatively more costly; (v) many times, minor improvement brought by this technique fails to bring an identifiable change in the phenotype of the new variety which may create problem in the registration of the variety; and (vi) the technique is not very successful in improving agronomically important quantitative characters. Therefore, the use of mutation breeding technique in improving crop species should not be overrated and this technique should be applied only under specific situations

1. When natural variability of any species is insufficient or lacks gene(s) for desired characteristics;
2. if the desired gene is tightly linked with some undesirable gene(s):
3. when some sort of compensating mechanism (strong negative correlation between component characters of grain yield, etc.) imposes a ceiling on the yield level and the biological system becomes helpless in further increasing the productivity through conventional recombination breeding;
4. if an outstanding cultivar succumbs to a new race of a pathogen and the breeder is interested to remove the defect of the cultivar without disturbing its superior genetic background;
5. when a negative correlation exists between a quality trait and disease resistance;
6. when the objective of the breeder is to change or modify some biochemical pathway in a crop species.

Fortunately or unfortunately, some of the specific situations mentioned above, are very well found in wheat. For example, genetic variability for *Karnal bunt* (KB) or *partial bunt* (a smut disease caused by *Tilletia indica* fungus) is low. The disease has considerably threatened wheat consumption in several parts of the world (India, Pakistan, Nepal, etc.). Therefore, mutation breeding has a good scope for widening the spectrum of genetic variability for controlling this serious disease. Similarly, there is a strong negative correlation between two most important component characters of yield, namely, grain weight and grain number per ear. If the wheat breeder tries to increase the value of one component character, the value of the other character is automatically reduced.

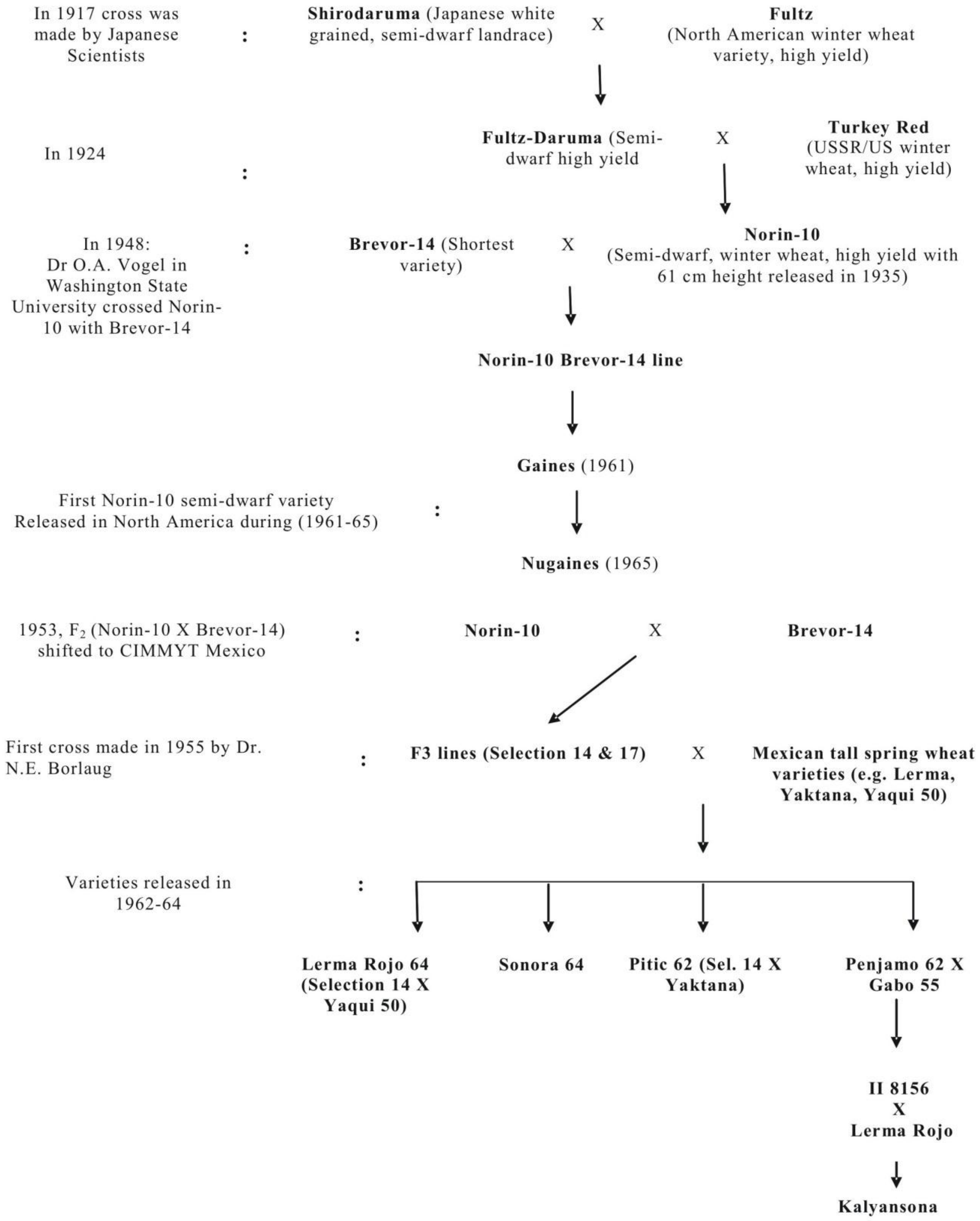

Fig. 2: Origin of semi-dwarf wheat

Some attempts have been made to increase variability of one component trait through artificial mutagenesis while keeping the value of the other component character constant. Furthermore, there is a strong negative correlation between lysine content and total protein content in wheat. A change in biochemical pathway may probably help in increasing the lysine percentage while keeping the total protein content constant. However, it should be kept in mind while using mutation technique in any plant breeding programme that this technique is considered as complementary to other procedures rather than as an alternate method to conventional breeding methods[17].

Mutant Varieties Produced

There are a number of examples where wheat cultivars have been improved through direct induction of mutations. For example, in India, the variety NP 799 (an awnless wheat variety) was treated with X-rays and new mutant variety NP 836 (with presence of awns and higher yield) was released in 1961. Similarly, *Sharbati sonara* is a mutant version of Sonora 64A. The variety *Sharbati Sonora* which has amber grain colour and was released in 1967, was developed by irradiating Sonora 64A with gamma *rays.* A third variety *Pusa lerma* is a mutant form *of* Lerma Rojo. This variety was approved for release in- 1971 and has amber grain colour (an improved characteristic).There are many examples of wheat cultivars developed through direct mutation breeding in countries. In Germany, hexaploid wheat (*T. aestivum*) materials were treated with X-rays in 1950s and were subsequently screened for reduced height and lodging resistance. Two mutant varieties namely, Els (approved in 1960) and Sirius (approved in 1968-1974), possessing desirable characters were released. In USA, two varieties, *Lewis* and *Stadler* (both approved in 1964 were developed using thermal neutrons; in Japan, the variety *Zenkouzi-komugi* (approved in 1969) was developed through gamma rays treatment; in USSR, the variety *Novosibirskaja* 67 (approved' 1970), was evolved after giving gamma rays treatment; and in Finland, the variety *Hankkijas* (approved in 1970) was developed using gamma rays. All these mutant varieties belong to *T aestivum* and possessed both the desired characters mentioned above.

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