Breeding strategies for the improvement of sunflower

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Sunflower is well known as an important oilseed crop and has been subjected to improvement by plant breeders, resulting in the yellow revolution in many countries. Russian plant breeders improved the oil contents of sunflower converting this crop from a roadside plant to a world-famous oilseed crop.

The cultivated germ plasm retained 50% of genetic diversity present in wild relatives. This may be threatened due to worldwide hybrid cultivation (*Table 1*) which share common parentage and sources of cytoplasmic male sterility. There is thus a need to use the available genetic diversity within cultivated and wild germ plasm, to develop pre-breeding lines and elite breeding material with good combining ability.

Sunflower breeding revolves around the development of breeding lines suitable for hybrid breeding, diseases, abiotic stress and herbicide resistance. These objectives were fulfilled by recurrent selection for population improvement.

Wide crosses were made to transfer cytoplasmic male sterility, diseases, abiotic and orobranchial resistance. Moreover, induced mutations were used to induce new genetic variability for diseases and herbicide resistance, and reduction of plant height. Marker-assisted selection has been validated for rust resistance, downy mildew resistance, oleic acid contents and fertility restorer genes.

Breeding objectives

Hybrid breeding

The hybrid vigour and heterozygous genetic base of hybrids allow them to show better yield even under stress conditions. The superior performance of hybrids in several studies, were due to the manifestation of heterosis.

Heterosis is defined as the superiority of F1 over the mid-parent value or better performance of F1 over the superior parent or best commercial variety. Heterosis is manifested due to genetic divergence of the parents, and superior combining ability of both parents causing the combination of diverse alleles in a single genotype. There was a positive relationship between genetic distance and best parent heterosis (Hladni *et al*., 2018).

In contrast to the open pollinated varieties (OPVs), which only exploited additive gene action for the improvement of plant traits, the performance of a hybrid is dependent on both additive and non-additive gene action. Some of the plant traits such as oil content, days to maturity, biotic or abiotic factors are fixed during the inbred line development through pedigree selection, and may be controlled through additive alleles.

Allelic or non-allelic interaction may be broken to release additive alleles through recurrent selection. However, the grain yield potential of hybrids is based on over-dominance, a genetic phenomenon in which deviation from mid-parents' value greatly exceeds to both parents.

Hybrid seed production in sunflower is done using a cytoplasmic male-sterile female line and fertility restorer male

Table 1: Worldwide examples of sunflower germ plasm resource collections.

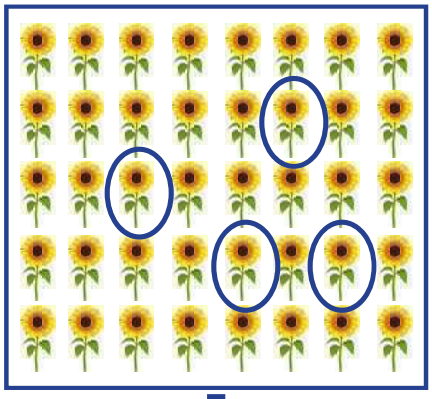
Institute	Collection
United States Department of Agriculture	4 087 accessions: 1 886 cultivated, 2 201 wild accessions.
French National Institute for Agricultural Research Occitanie-Toulouse, France	5 576 cultivated accessions, >500 wild ecotypes.
The Indian Institute of Oilseeds Research, India	3 273 accessions: 1 200 exotic lines, 97 genetic stocks, 360 inbred lines, 42 wild species and 154 wild species derivatives (Dudhe and Sujatha, 2016).
The Vavilov Research Institute of Plant Industry, Russia	2 230 cultivated sunflowers and 550 wild accessions (Gavrilova *et al*., 2014).
Chinese Academy of Agricultural Sciences Youliao Institute	2 813 accessions: predominantly cultivated (Gao *et al*., 2001).
The Institute of Field and Vegetable Crops, Novi Sad, Serbia	Several thousand lines (S Terzic, personal communication, 2016), wild sunflower collection of just over 1 000 accessions of 47 wild species, (Atlagić and Terzić, 2015).

Figure 1: Procedure for Pustovoit's famous 'method of reserve' to develop high oil contents progenies.

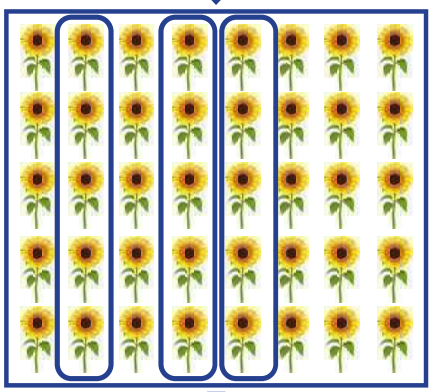


10 000 to 15 000 plants are selected, having 500 to 2 000 seeds per plant.

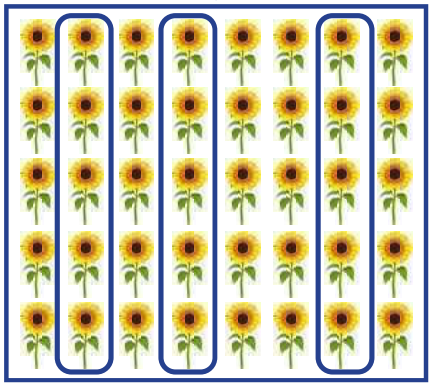
Seeds are analysed for hull and oil contents to further select 1 200 to 1 500 heads.



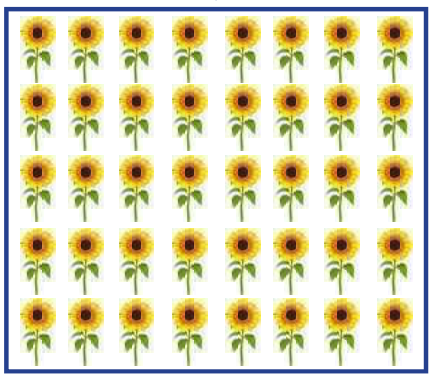
Heterogenous population, a collection of elite cultivars, experimental hybrids and germ plasm accessions.



Progeny evaluation of selected plants with high oil contents against checks as control for agronomic, diseases and quality.



15 to 20% S0 selection is planted in second-year nursery. Remnant half-sib seed of year 1 is also used to test for disease resistance.



25 to 5_0 S0 plants of season 1 are planted for cross-pollination and random mating. Undesirable plants with tall and branched plants are rejected. Obtained seed is used to test oil contents and seed is bulked to constitute the next cycle of selection.



The diversity in ray and disc floret colour of sunflower. (A) Crimson red ray floret with brown disc floret. (B) Yellow with crimson red pattern ray floret with purple disc floret. (C) Yellow wide ray floret with yellow disc floret. (D) Yellow narrow ray floret with green disc floret. (E) Yellow ray florets with purple disc floret. (F) Crimson red with yellow tip ray floret and purple disc florets. (Photographs: Saeed Rauf)

line at a ratio of 4:2, and pollination is facilitated through honeybee hives (Greenleaf and Kremen, 2006).

Oil content

The improvement of oil content is a major objective of sunflower breeding. Oil content entails quantitative inheritance traits and genetic variation was affected by additive genes. Oil content has ranged from 30 to more than 50% and has shown tremendous improvement due to selection by plant breeders.

Historically, oil content was subjected to improvement during the earlier part of the 19th century through a method of seed reserve proposed by Pustovoit (*Figure 1*), which led to the development of high oil content lines. Seed morphological traits such as embryo size and testa thickness were important traits affecting oil contents. Smaller seed size with larger embryo and thin testa gave higher oil recovery (Rauf *et al*., 2017).

Improvement in oil content occurred due to higher kernel-to-achene ratios (Pereira *et al*., 2000). Oil content accumulation was not constant during the grain-filling period. Oil content accumulation rate was slower after anthesis but increased over time and became constant during the physiological maturity (Rondanini *et al*., 2003).

Oil concentration was also dependent on the size and concentration of oil bodies. However, high oil content lines had a high concentration of oil bodies rather than larger oil bodies. Oil bodies are the storehouse of triglyceride containing a single layer of phospholipid, which is made intact through oleosins and caleosins proteins (Murphy, 2001). The range of oil body diameter was 0,65 to 2µm in various crop species, including sunflower.

The accumulation rate of oil bodies was negatively affected by the protein contents. However, oil content is significantly affected by the environment, and medium heritability (0,57) of the trait was estimated over multiple environments (Mokrani *et al*., 2002). Several QTLs (quantitative trait loci) have been identified on various linkage groups for seed oil contents. These QTLs had additive to dominant effects and were closely related to domesticated traits in sunflower (Leon *et al*., 2001; Burke *et al*., 2005).

Rust resistance

Rust caused by Puccinia helianthi Schwein is one of the major factors affecting yield and quality of sunflower seed. It causes a reduction in the green leaf area which reduces the overall assimilation of photosynthates and translocation. It also causes a reduction in the seed weight, size and oil content.

Rust thrives in warm and humid conditions, and fungicides can be used to control the disease, but this method is expensive and challenging. Therefore, incorporating rust resistance in inbred lines is the preferred method to control this disease.

Several rust-resistant genes (R1, R2, R4u, R5, R12, R13a and RHAR6) were identified in various breeding lines, varieties and restorers. A single source of resistance is not sufficient due to the emergence of virulent races which may defeat a single source of resistance in sunflowers. Molecular markers have been developed to stake these genes in single genotypes which increase durability and the spectrum of resistance (Paniego *et al*., 2012).

Two genes were recently identified in HA-R6 and RHA-397 which provide resistance against all virulent races of rust. Pedigree and marker assisted selection was carried out to develop two inbred lines (HA-R12 and HA-R13), which contained multiple rust-resistant genes. HA-R12 contained the rust-resistant (R) genes, R2 from MC29 (AUS) and R13a from HA-R6. HA-R13 contained R5 from HA-R2 and R13a from HA-R6.

Application rust-specific markers confirmed that HA-R12 and HA-R13 contained two rust-resistance genes in the homozygous condition, with both lines showing high levels of resistance to rust races 336 and 777, which are the most virulent races (Ma *et al*., 2016).

Heat stress

Global warming has caused a rapid rise in air temperature. Higher temperatures cause increased leaf senescence, early maturity or premature phenological development due to higher accumulation of heat units in plants (Kalyar *et al*., 2013). It causes higher gametophytic sterility and reduces grain filling. It also accelerates the impact of other stresses such as water and salinity.

Sunflower showed the highest growth at 27°C, and temperatures higher than 30°C were unknown to induce temperature stress. Plant phenological traits such as downward head position and erect leaves reduced pollen sterility and lower canopy temperature. Both traits were selectable in segregating

populations and had medium-realised heritability (Kalyar *et al*., 2013).

Breeding methods

Conventional breeding procedures

Sunflower is a cross-pollinated species and all breeding methods of cross-pollinated species such as recurrent selection methods, are applicable for the population improvement programme. However, pure line selection methods are applicable for the development of inbred lines with the additional step of combining ability analysis of inbred lines. The backcross method is used to intogress disease-resistant or monogenic traits.

Inbred line development

Superior inbred lines are obtained by crossing appropriate breeding lines such as crossing disease-resistant or drought-tolerant inbred lines with highly fertile, high oil content and early maturing lines. Disease-resistant or drought-tolerant lines are generally developed from interspecific crosses, which are poor in agronomic characteristics such as high plant height, low oil content and late maturing (Hussain *et al*., 2017; Shehbaz *et al*., 2018).

Resistant genes may be transferred to the elite lines through backcross methods, or segregating populations may be developed for the pedigree selection in subsequent generations (Shehbaz *et al*., 2018). Pedigree selection was generally carried out for traits having high additive genetic variance (Kalyar *et al*., 2013a).

Heritability in the narrow sense or realised heritability are indicators of a selection response. Oil quality traits such as tocopherols and oleic acid contents have been transferred in elite germ plasm through a backcross scheme (Jonic *et al*., 2000).

Traits such as oil content may have been subjected to the pedigree selection for the development of high oil content inbred lines. Generally, five to six rounds of selection (F2 to F6) are sufficient for improvement and fixation of characteristics in inbred lines. Traits related to heat resistance such as downward head orientation at the time of anthesis, have been used as selection marker for the development of heat resistant inbred lines (Kalyar *et al*., 2013a). These selections led to the development of some superior heat resistant hybrids (Khan *et al*., 2017).

Morphological traits such as lower leaf area and cuticular waxes have been used as marker traits for the selection of drought tolerant inbred lines in segregating generations (Hussain *et al*., 2017; Hussian *et al*., 2018).

Combining ability analyses

This is an important step in the evaluation of sunflower breeding lines for their performance in hybrid breeding. Combining ability is the ability of a breeding line to produce the superior progeny upon crossing with testers. A tester may be underperforming if the aim is to uncover deleterious or recessive alleles carried by the female lines (Kalyar *et al*., 2013b). However, superior lines may be crossed to determine the best specific combiners or cross combination, which could be used for the development of high performing hybrids (Khan *et al*., 2018).

In vitro techniques

Sunflower inbred line development requires six cycles of self-pollination and three to four years to achieve homozygosity. It would further take one more year to test the combining ability of the developed homozygous lines. In vitro or in vivo (doubled haploid inducer lines) methods of haploid line development can reduce the time required to achieve homozygosity.

Once the protocol for the development of haploid line is optimised, the required homozygosity can be achieved in a single year. The anthers collected between the dyad and tetrad stages were found to be more responsive for the haploid induction in sunflower. Anthers that were pre-treated with high temperature treatment (35°C) for twelve days were found to be more successful.

Generally, half-strength, medium supplemented with vitamins of Morel and Wetmore with vitamin B12 and a mixture of amino acids, 120g L-1glucose, pH 5,9 plus 0,5g L-1 naphthalene acetic acid, and benzene amino purine, were considered for haploid plant regeneration from anthers culture (Mezzarobba and Jonard, 1986).

A sunflower embryo rescue technique has been used to excise interspecific embryo and to culture over the growth media after excision from the developing embryo. Seven-day old embryos (*Helianthus annuus* × *Helianthus mollis*) were excised from mother plants and were inoculated over the Murashige and Skoog (MS) media (Faure *et al*., 2002).

In vitro multiplication through nodal culture of interspecific hybrid (*H. annuus* × *H. simulans*) was done on MS media supplemented with 0,5mg L-1 benzyladenine (Prabakaran and Sujatha, 2004). Immature embryo cultures were done to shorten the life cycle and subsequent generation advancement of the crosses. Embryos were excised after ten days post-pollination and inoculated over simple MS media (Dagustu *et al*., 2010).

Most of the cultured embryos were regenerated into vigorous seedlings with three to six leaves (Dagustu *et al*., 2010). In vitro screening for drought tolerance was carried out on half-strength MS media supplemented with osmotic 5% polyethylene glycol (PEG-8 000) to reduce the osmotic potential of the media (Khalil *et al*., 2016).

An amphidiploid between cultivated and perennial wild sunflower was regenerated through somatic embryogenesis (Fu *et al*., 2017). Somatic embryogenesis has been optimised using various formulations and concentration of media and growth regulators along with immature embryo size (Sujatha and Prabakaran, 2001).

The highest induction of somatic embryogenesis occurred at Gamborg basal salt media (120 to 210g L–1) sucrose, 0,8 to 1% agar, smaller-sized embryos (0,5 to 2mm) and at incubation temperature of 28 to 32°C.

Growth regulators such as 2,4-D promoted direct embryogenesis, BA+NAA facilitated formation of single/multiple shoots, while there was no response on 2,4-D+kinetin SM (Sujatha and Prabakaran, 2001).

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