

**Canola Genetic Engineering for Long-Term Agriculture and Global Food Security**

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Corresponding email - nitu.agri@cumail.in**Abstract**

Throughout the globe, abiotic stress is a serious threat to agriculture. Moreover, the extreme climatic conditions like drought, flooding, global warming and others are the major yield-limiting factors for crop plants and impacts forceful challenges to global crop production. Contribution of *Brassica Napus* is more towards the oilseed industry and has also achieved worldwide acceptance. It is the third most important source for vegetable oil for human consumption after palm and soybean oils. Canola crop is widely used as vegetable oil and meals for animals and is one of the first genetically modified crops to reach commercial markets of Canada. Genetic modification is required to protect these kinds of crops from various kinds of stress such as metal stress, extreme climatic conditions, drought and saltiness. In this review, we have discussed the current and future role of genetic engineered canola to sustainable agriculture systems.

Keywords: Genetic modification, Canola crop, abiotic stress, environment, global warming

Introduction

Plants face adverse environmental conditions to their growth and development. These disruptive environmental conditions are in the form of various abiotic and biotic stresses. The opportunity provided by genetically modified crops is to increase in yield of plant and increasing its nutritional values can be seen in a shorter period (Guardian *et al.*, 2016). Climate change conditions, may exposed plants to many abiotic stresses including high/low

temperature, drought, salinity, heavy metals etc. These stresses cause the molecular, biochemical and physiological changes in the plant. Plants are subjected to many challenges at the same time in the field, making it difficult to generate stress-tolerant plants.

The population is increasing day by day and it is expected to increase 9 billion by the end of 2050. To full field the demand for food world agriculture need to be rise and develop stress resistance plants. Uses of biotechnology

techniques in agriculture like transgenic techniques, often seem to be called as genetically engineered plants, are among the most common technique as they allow the transfer of genes between species, resulting in genetically modified organisms (GMOs) that causes an arguable issue because plant generated doesn't have biological antecedents (Grey R. *et al.*, 2001)

Canola (*Brassica napus*) is an oil seed-based crop grown on approximate 31 countries in the world (FAO 2009). The allotetraploid formation of *Brassica napus* is done by hybridization or mixing of *B. rapa* L. and *B. oleraceae* L. which can also be combined with more than 15 other mustard species (Fitz John RG *et al.*, 2007). A number of these mustard species are also considered to be poisonous weeds. Canola oil is said to be useful vegetable oil which is used in a range of cooking purpose and making of meals (PLANTS database USDA *et al.*, 2011.) Canola crop has been recognized as one of the world's most useful and established oilseed crops and the most earned food crop commodity (James C, *et al.*, 2015). It's low in saturated fats and therefore is accepted around the world. However, there are markets which are looking for good oil which should have health benefits and also must be unique

(guardian *et al.*, 2016). Before less in a number of erotic acids levels which leads to use of this oil for both industrial purpose as well as for food (Southgate EM, *et al.*, 1995).. Canola has been taken as a good source of energy because of having its different features like having omega 3 in it which makes it different to other food crops, hence also used in biodiesel production (James C *et al.*, 2016). Breeding through conventional methods takes a lot of time to grow and develop plants (Delaney *et al.*, 2015). In fact, by 2050 it is estimated to feed sufficient food to about 9.7 billion people (FAO 2017) and by 2100 approximately 11 billion people are considered to be fed (James *et al.*, 2015) which should be considered as one of the major challenges of this century. A great opportunity has been provided by Genetically modified (GM) crops to increase the yield of this plant for food production; which can be seen in a shorter period (Gaurdian *et al.*, 2016). These types of crops also offer the possibility to increase in size for the available genetic pool for reproducing by overcoming various sexual conflicts between plants. In this review, we have discussed the current and future role of genetic engineered canola to sustainable agriculture systems.

Table 1 Rapeseed Production by Country demonstrating the Worldwide Production of Meal, Oil, and Oilseed by Country.

	Meal, Rapeseed			Oil, Rapeseed			Oil Seed, Rapeseed		
COUNTRY	2013- 2014	2014- 2015	2015- 2016	2013- 2014	2014- 2015	2015- 2016	2013- 2014	2014- 2014	2015- 2016
Thousand metric tons									
China	11631	11505	10975	6579	6500	6195	14458	14600	14100
India	3720	3250	3600	2400	2100	2320	7300	6310	7150
Canada	3925	4150	3945	3050	3230	3070	18551	16410	13300
Japan	1353	1360	1360	1054	1075	1075	2	2	2
European Union	13780	14370	13398	9946	10371	9670	21304	24394	21300
Other	5026	5586	5198	3479	3837	3641	10473	10193	8840
World Total	39435	40221	38476	26508	27113	25971	72088	71909	64692

Origin of Canola their nutritional values

Originally from the Mediterranean area and Northern Europe, *B. napus* is commonly known as rapeseed. Rapeseed was identified in 2000 BC as a high-erucic acid crop, containing >40% erucic acid in the oil.

Canola seeds contribute approximately 13% of the total oilseed and protein meals production in the world. The production industry for canola and rapeseed is increasing because of the increased demand for vegetable oil in China and India and for the production of

biodiesel and its use in the US (USDA, 2011).

Canola seeds have been genetically modified from traditional varieties of rapeseeds by plant breeders to obtain plants with low levels of erucic acid in the oil and low levels of glucosinolates in the non-oil part of the plants. Therefore, by the definition, rapeseeds that contain low levels of erucic acid (< 2%) in oil and glucosinolates (< 30 µmol/g) in a defatted meal are called canola in North America, but they are called “double-zero” or

“double-low” rapeseeds or 00-rapeseeds in Europe.

Due to this kind of discoveries, rise canola populations or non-engineered populations expressing biotechnical characteristic which are reportable from Japan, Australia, Great Britain(Pesselet *al.*, 2001)(Aonoet *al.*, 2006). In 1998, GE canola crop was 1st approved for business and unfair means in the United States and currently, more than 90% of the expanse planted within the U.S. nation is genetically modified for designing weeds resistance crops of canola (Sinimus 2009). Since 1969 Canola crop has been full-grown in Australia's southern grain belts. During 2010-11, Queen Victoria has made around 476000 tons of canola seed crop with an approximate worth of 293 million dollars. However, Canola has additionally encompassed a useful result on a wheat crop by reducing diseases caused by soil on crop. Once it's incorporated as a part of the crop rotation system. The origin of canola was related to the trademark of rapeseeds of North American countries. It originates from the foothills of the Himalayan range;

canola most likely originated within the Mediterranean Sea region and which causes the results of natural crossing between these 2 species *Brassica Campestre* and *Brassica oleracea* crops. Canola was developed in North American country within the early Nineteen Seventies through standard plant breeding for its oilseeds and is currently most preferred edible oil in most parts of Canada. It's a part of the family Cruciferae, and herbicide resistance or unwanted plant tolerance which has been predominately introduced into rapeseeds, though alternative species which embrace *B. rapa* and *B. juncea*.

Decrease in productivity due to abiotic stress and genetically modified Canola

Abiotic stress affects the growth and development of crops which leads to global crop loss. This harmful effect of abiotic stress causes a huge amount of economic loss. Various genetic modifications have been done in the canola plant to make it resistant to various stresses. For making canola resistant from various stress we need to make it transgenic. These stresses have been described in figure 2.

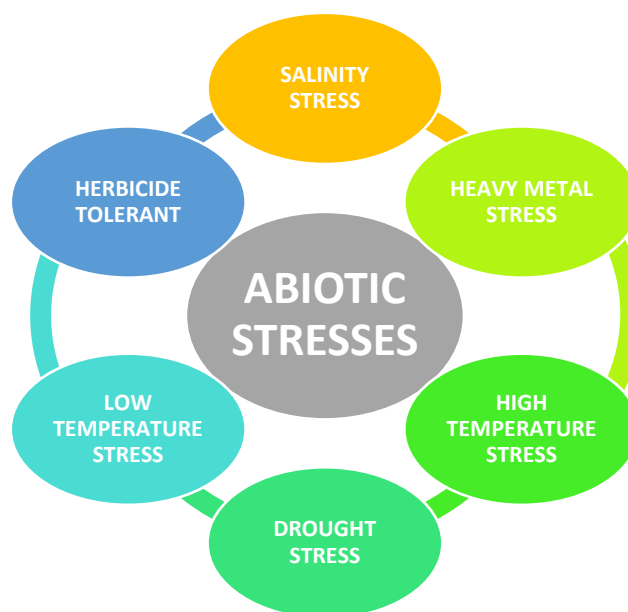


Fig 2: Different types of stresses

Herbicide-tolerant and canola

Herbicides or weed killers are the essential substrates for weed administration in canola crop (Harker *et al.*, 2013). Initially, crop growers are usually depended closely on pre-plant-incorporated weed killers. However, an increasing reliance on post-emergence herbicides for weeds control has a great deal. In canola, incremental weed management gains have paralleled the development of new herbicide technology. Initially, di-nitroaniline herbicides provided control of major weeds such as wild oat. This nature of this crop has authorized the enormous acquire decrease for the creation of herbicide-tolerant canola cultivars, including genetically modified (GM) canola (Beckie *et al.*, 2011). The repetitive use of any one type

of herbicide on giant populations of genetically variable weed species are selected for resistant weed biotypes. Herbicide resistance is now enormous in annual ryegrass with 12 different weeds species throughout the canola growing areas of Australian region (Heap *et al.*, 2009). The software of each non-recombinant and recombinant DNA technical homologies to canola has been produced herbicide-tolerant canola cultivars (Raman *et al.*, 2018). Due to the fast adoption of these cultivars allows the use of broad-spectrum herbicides being supplied to farmers with greater flexibility to manipulate weeds with better yield and economic returns (Beckie *et al.*, 2011). Conventional canola is tolerant of various varieties of herbicides.

Through cause and sequence transferring of plant breeders have developed canola that's tolerant to specific herbicides or teams of herbicides through mutagenesis, genetic modification or gene transfer methods; which is as follows:

- a) Triazine-tolerant canola (TTC) has been produced by to allow farmers for growing canola on fields. Certain weeds like a mustard plant, stinkweed, ball mustard and other varieties can't be controlled by herbicides in fields of standard canola. TTC varieties yield is less than that of the standard canola varieties below weed-free conditions. Triazine-tolerant canola was produced at the University of Guelph, with the *B. napus* selection, OAC Triton, and its database is submitted in 1984.
- b) The primary imidazolinone-tolerant (Pursuit + Odyssey herbicides) *B. napus* selection "45A71" got patented in 1995. These selections contain a tolerance attribute that was developed and can be caused by cyanamide (BASF). The primary transgenic *Brassica napus* selection QUEST. In 1995 Monsanto produced and acknowledge as Quest which shows tolerance to the weed resistant glyphosate.
- c) In 1995, Innovator and Independence were registered. These varieties were produced by Aventis (transgenic *B. napus*) contains

a sequence that provided resistance to the weed resistant glufosinate-ammonium.

Salt stress and canola

Salinity stress is recognized as one of the most crucial abiotic stresses that inhibits growth and development of plants especially in dry and semi-dry atmospheres during their flowering and pod stages (Yamaguchi and Blumwald2005). High level of saltiness prompts disruption in sprouting of flowers and inappropriate growth of the plant. (Singh 2015). It is estimated that half of the world's land area will become saline by the time of the 21st century (Mahajan and Tuteja *et al.*, 2005). Soils with inappropriate degrees of saltiness have a low water-holding limit/capacity subsequently, it is not easy for the plant to retain water and it's supplements. (Agarwal *et al.*,2013). One of the major important outcomes of osmotic weight on vegetation is the generation of responsive oxygen species (ROS) in gigantic sums that went with through oxidative harms, for example, the debasement of proteins, lipids and DNA (Das and Choudhury R, *et al.*, 2014). Plants creating on saline stipulations take up unsafe particles, specifically Na⁺ and Cl⁻ions. Collection of Na⁺ and Cl⁻ particles in enormous sums is harmful to the cell and mixes osmotic pressure (Agarwal *et al.*, 2013). These particles upset film uprightness, cell digestion, protein structure, metabolic

development, and photosynthesis (Ghosh and Xu 2014). Even though blooms have a scope of approach the withstanding pressure, broad loss of yield transpires. In the interim, there is a consistently expanding request to deliver enough dinners for the world's developing populace (Deinlein *et al.*, 2014) (Singh 2015). To deliver these difficulties to the world's nourishment security, the building of vegetation or nourishment of crop has done to make species that can endure saltiness and has been considered as an assured methodology. Accomplishing salt-resistance in vegetation requires the total ability of plant sub-atomic instruments and its impact to salt resistance. Like diverse fundamental crops, salt pressure lessens yield of canola and its creation. Some canola cultivars show high resilience to saltiness, but others are not (Bandehaghet *al.*, 2018) (Eyvazlouet *al.*, 2019) (Bandehaghet *al.*, 2011) (Gharelo-Shokri *et al.*, 2016). Regardless of different considers that has been continuing for canola crops cultivars underneath salt pressure, development in building tolerant canola has been moderate, comprehensively talking because of the reality of the unpredictability of the atomic systems complexity and the absence of enough data. Saltiness expands poisonous quality in the dirt which prompts the devastation of several helpful minerals and nourishing nutrients that are fundamental for its development.

Heavy metal stress and canola

Now a day's agricultural practices for example mining, industrialization are some anthropogenic things to do which we can see creating a continuing dangerous effect on our environment. And basically, due to these factors the possibilities of heavy metal retention have increased in air, soil and water. Due to an increase in heavy metal contaminated soils, these environmental contaminations impart toxic into the soil that affects animals as well as plants. As plants respond to these harmful conditions, their flora evolves to a complex mechanism (physiological or molecular) for high tolerance power, adaptation with the environment and increase in their life span. Several techniques like transgenic technology biofortification and traditional breeding are initially developed for heavy metal stress-resistant formulas which however required skilled labour for these practices and also, they are very time taking processes. Interestingly, we can use microorganisms as another technology for increasing metal tolerance inside the plant. For various crop improvement or crop management practices these microbes are being used for the protection of crops from heavy metal stress. Soil-borne microorganisms in contact with various crops play a very important or necessary role in the adaptation of vegetation to various extreme

environments, and can, therefore, these kinds of microorganisms can be used for improving microbe-added metal tolerance in the crops. Plant associated microbes limit metal concentration in soil and reduce metal availability in crops. Various researchers have taken in a role in account to aware various countries to understand the dangerous effect of metal stress, their role of plant-associated microbes in metal stress tolerance and their signaling response to metal stress. In addition to highlighting the opportunities and challenges in this persisted region of lookup on plant-microbe-metal interaction (Tiwari and Lata2018). As the terrestrial ecosystem is decreasing, stress caused by heavy metals becomes a major concern. This happens due to excessive industrialization. Heavy metals such as Cu, Co, Zn, Mn, and Ni are indispensable for critical biotechnical tactics and developing pathways.

CRISPR technology in the field of canola

CLUSTER REGULATORY INTERSPACED SHORT PALINDROMIC REPEATS (CRISPR) genome editing is an efficient method for crop improvement. The discovery of CRISPR was a revolutionary step in genome editing. This edging technology progressively involves the inclusion of Cas 9, which binds to guide Ribo Nucleic Acid (gRNA) of around twenty nucleotides correlative to the Deoxyribose Nucleic Acid

stretch inside the objective quality. The abbreviation Cluster Regulatory Interspaced Short Palindrome Repeats, first began in 2002(Jansen *et al.*, 2002) alludes to pair rehashes to the edge by non-redundant Deoxyribose Nucleic Acid that was extended and was first seen in the last steps of *Escherichia coli* qualities. (Ishino *et al.*, 1987). These non-monotonous groupings were seen as homologous with outside DNA arrangements got from plasmids and bacteriophages in 2005. In this way, the component of homology-subordinate cleavage was investigated for genomics alteration and the innovation of Cluster Regulatory Interspaced Short Palindromic Repeats /Cas9 cleavage 'landed' as a promising genomic alteration apparatus (Mojica *et al.*, 2005).

The Cluster Regulatory Interspaced Short Palindrome Repeats cleavage technique requires (i) a very small manufactured guide RNA grouping mainly twenty nucleotides that quandary the objective DNA and (ii) its cas9 nucleases protein which separates 3–4 bases after the protospacer adjoining topic PAM by and large 5' NGG (Jinek *et al.*, 2012). The nuclease protein i.e. Cas9 is made out of two spaces, (a) RuvC-like areas and (b) an HNH space, with every space cutting one DNA strand. Following the advancement of the CRISPR associated with a cleavage system, it must be generally

applicable in plants and creature genomic alteration. Somewhere in the range of 2010-2018, almost 5k articles have been distributed specifying the utilization of Cluster Regulatory Interspaced Short Palindrome Repeats. Executing a CRISPR venture includes straight forward advances in other words (i) distinguishing the Protospacer adjacent motif succession in the objective quality, (ii) combining a solitary guide RNA (small guide RNA), (iii) cloning the small guide RNA into an appropriate paired vector, (iv) presentation into have species/cell lines change pursued by (v) screening and (vi) approval of altered lines. A few stages engaged with Cluster Regulatory Interspaced Short Palindrome Repeats /Cas9 interceded genome altering (CMGE) permits even a very little research centre with a constrained plant change set up to do different genome altering ventures. Cluster Regulatory Interspaced Short Palindrome Repeats /Cas9 systems have been utilized all the more broadly to alter plant genomics in the last 50 years contrasted with ZINC FINGERNUCLEASES and Transcription activators like effectors nucleases and are intelligent of its simple to deal with.

Conclusion

It has been observed that plants which are being genetically modified give out better

yield, healthy growth and enhanced tolerance in abiotic stress as compared to non-genetically modified crop. Development of stress resistance shows bigger transgenic canola plants gives solution for crop improvement & production. It has been accepted in various countries now because of higher yield and better quality. As the blooming of flower increases their seed content also increases resulting in a higher range of canola oil for commercial use. A transgenic plant provides yet to be famous in major countries for cultivation. As an increase in the rate of cardiovascular diseases, there is a need for shifting to a better variety of oil for daily consumption. Canola is one of them because of its lower uric acid content and presence of omega 3 in it.

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