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Shattering tolerance in *Brassica napus* L.

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ABSTRACT

Brassica is the second-largest oilseed crop after Soybean. The total production of Brassica in the overall world is 71 million tons. In Pakistan, its total production per unit area is very low. Biotic and abiotic stresses mainly affect the brassica crop. In agriculture, shattering is the dispersal of crops seeds before their ripening. The pod wall shatters and breaks apart when it loses its hydration and cells split in a dehiscence zone organized at a suture between the edge of the lignified pod and the vascular tissue replum. The degeneration of middle lamella and loss of cellular cohesion in the dehiscence zone are the main reasons for pod shattering and seed losses. Grain yield losses in Brassica vary from 10 to 25 percent due to shattering. More than 400 kg has-1 or 12% seed losses can be occurred due to pod shattering under unfavorable conditions. Insect pest and disease damage also accelerate ripening and pod shattering. The main breeding techniques for developing rapeseed grain yield potential are a good knowledge and application of the morphological, physiological, and genetic basis of grain yield. Modern technologies, such as embryo rescue, marker-assisted breeding, and novel variation (mutation), may make it much simpler to introduce new rapeseed types having shattering tolerance than traditional methods. Thus, an overview of anatomical and physiological aspects and genetics of shattering is presented in the context of recent advances in molecular genetics and several agronomic managements to avoid shattering in Brassica.

Keywords: *Brassica napus, Shattering resistant.*

INTRODUCTION

Brassica is the second-largest oilseed crop after Soybean (Raymer, 2002). It belongs to the family Brassicaceae. This family includes 25 tribes with about an additional 5 are under study (Al-Shehbaz, & Beilstein et al., 2006). The genus Brassica and its wild relatives of the Brassicaceae family is made up of 48 genera and approximately 240 species (Warwick & Hall, 2009). There is a total of 100 species out of which four species are most widely cultivated i.e., *Brassica rapa*, *Brassica napus* L., *Brassica juncea* L., and *Brassica carinata* L. These Brassicas generally derived from the two species *B. napus* L. and *B. campestris* L. (Gupta & Pratap, 2007).

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It is confirmed by the studies of proteins and DNA that there are three basic genomes of the Brassica family. These three genomes make the oilseed crop species and tetraploid vegetables. Climate change is very crucial for the security of food especially in developing countries like Pakistan. Almost 38.5% of its population is related to agriculture and contributes 19.3% to the GDP of Pakistan. The total production of Brassica in the overall world is 71 million tons. But in Pakistan, its total production per unit area (349 thousand hectare) is very low. Its average yield per hectare is 785 kg (Go, 2019).

The oilseed crops are very beneficial for mankind. Especially in the case of human health it reduces the risk of heart disease, helps to boost up the immune system, helps to promote cell growth, helps to improve the body metabolism, helps in curing acute pancreatic, and most important is its oil has Antibacterial properties. It also lowers anxiety and depression. Brassica is mainly used as an oil for cooking and its protein as meal cakes for livestock feed. Almost every part of the Brassica family is useful. Including roots (rutabaga, turnip) flowers, (cauliflower, broccoli) stem, (kohlrabi) leaves(cabbage), buds (brussels sprouts) and seeds (rapeseed). The brassica group consists of high amounts of vitamin C, Vitamin K, Mn and soluble fiber (Nugrahedi, & Verkerk et al., 2015)

Pakistan has been constantly chronically deficient in its production. The main reason for growing the oilseed crops is to narrow the gap between consumption and production of edible oil. The main reasons for this gap are low soil fertility, imbalanced use of fertilizers, lack of advanced machinery for sowing and harvesting, and use of non-certified seeds. But recently oilseed attracted more attention due to increasing demand for healthy vegetable oils, biofuels, pharmaceuticals, and other oleochemical uses.

In agriculture, every crop faces a lot of problems. There are two main stresses i.e., biotic and abiotic which mainly affect the brassica crop. Due to adverse changes in climatic conditions, abiotic stresses like drought, salinity, temperature, etc. mainly affect the brassica crop. During drought, there is a reduction in plant height, fewer photosynthetic pigments, a smaller number of branches per plant (Sadaqat, & Tahir et al., 2003). Reduction in Pollen germination and pollen tube length is due to high temperature in Brassica (Singh, & Kakani et al., 2008). Low temperature can cause a reduction in germination rate and seedling emergence. Whereas biotic stresses are like Alternariol Blight, Damping-off (Fusarium or Pythium species), Downy mildew (*Hyaloperonospora parasitica*), Leaf spot/target spot (*Alternaria species*), Powdery mildew (*Erysiphe cruciferarum*), White blister (*Albugo candida*), etc caused by special causal organisms. (Zheng, & Wilen et al., 1994)

The pod wall shatters and breaks apart when it loses its hydration and cells split in a dehiscence zone organized at a suture between the edge of the lignified pod and the vascular tissue replum (Morgan, & Bruce et al., 1998). Because to polygalacturonase's pectin degeneration, the dehiscence zone cell walls split along a line parallel to the middle lamella, resulting in the separation of the dehiscence zone cell walls. The pods open as they break off the vascular connections that travel from their wall to their replum via the dehiscence zone due to the usage of outside powers by interacting with other pods or racemes or the employment of collecting gear (Petersen, & Sander et al., 1996).

Shattering with its anatomical and Physiological Aspects:

In agriculture, shattering is the dispersal of crops seeds before their ripening. From agriculture, prospective shattering is an undesirable process in the history of crop domestication (Jiang, & Fu et al., 2013). Shattering is the detachment of pod valves (encloses the seeds) from replum. It could occur due to the impacts of other plants (under windy conditions) or due to the impact of harvest machinery (during windrowing). There is an occurrence of the abscission layer (consists of thin-walled and non-lignified cells) in the sutures of siliquae of susceptible

Brassica. But absent in the Brassica rapa types. These abscission layers are prejudiced to pod shattering (Kadkol, & Beilharz et al., 1986). The gene named INDEHISCENT (IND) reduced the level of auxins in the dehiscence zone of the layer and this reduction causes the development of the abscission layer (Sorefan, & Girin et al., 2009). In *B. napus* there is strong evidence for enzyme action (beta 1,4 glucanase) present in the abscission layer, results in the degradation of the middle lamella (Meakin & ROBERTS, 1990) (MEAKIN & ROBERTS, 1990). The dehiscence zone is the main factor for the shattering of pods (Ferrándiz, 2002). The degeneration of middle lamella and loss of cellular cohesion in the dehiscence zone are the main reasons for pod shattering and seed losses (Lersten & Carlson, 2004) (Bennett, & Roberts et al., 2011). According to the results, Brassica oleracea exhibited enhanced glucanase activity in DZ cells, which corresponded with cell wall degradation at the site of fruit dehiscence (Kemmerer & Tucker, 1994). The use of antibodies against -glucanase revealed that the infusion of antibodies completely reduced the break quality in the abscission zone, while the progressions of this chemical and its appropriation were completely associated with abscission forms, demonstrating that -glucanase is required for abscission (Sexton, & Durbin et al., 1980).

In some cases, hormones are also responsible for the shattering of plant cell walls. Ethylene, for example, has been linked to the processes of fruit ripening and abscission and has been found in the process of pod shattering (Abeles, & Morgan et al., 1992). The beginning of pod dehiscence has been linked to a spike in seed ethylene synthesis; furthermore, transitory peak ethylene production has been linked to an increase in glucanase movement, the enzyme responsible for the cell wall breakdown that leads to pod shattering (Oeller, & Lu et al., 1991). It has been shown that although ethylene does not induce dehiscence, it is responsible for speeding up the onset of senescence (John, & Drake et al., 1995). According to one research, gibberellic acid is required for the long-term elongation of pods in oilseed rape (Bouttier & Morgan, 1992). In addition, the presence of INDEHISCENT protein in Arabidopsis fruit is needed for the protein's functions to be carried out (Arnaud, & Girin et al., 2010).

Genetics of Shattering in *B. napus* and *B. Rapa*:

Current research priorities to understand the genetics of Shattering included the crops like Barley (Kandemir, & Kudrna et al., 2000), Buckwheat, Grain Amaranth, and Oilseed Rape (*B. napus* L.). There is less genetic variation in *B. napus* as compared to *B. Rapa*. There are 2-3 genes responsible for determining shattering in *B. Rapa* var yellow and var brown sarsoon in a cross with Torch. Dominant epistatic interaction was found in their F2 segregation pattern. The result that appeared after a cross of Torch×DS-17-D was additive, non-additive genetic variance, and high broad-sense heritability (Kadkol, & Beilharz et al., 1986). It is reported that there are mutant genes that cause alteration in pod anatomy in the shattering zone of Arabidopsis. SHATTERPROOF1 (SHP1) and SHATTERPROOF2 (SHP2) are two genes responsible for the shattering phenotype. While their double recessive shp1 and shp2 produce non-shattering and there is not only absence of abscission layer but also reduced lignification of margin cells (Liljegren, & Ditta et al., 2000). The development of the abscission layer in the dehiscent zone of Arabidopsis is due to gene ALCATRAZ (ALC). The mutant phenotype alc does not contain an abscission layer (Rajani & Sundaresan, 2001). Further research has shown that IND influences lignification of the valve margins and differentiation of the abscission layer by regulating auxins levels (Sorefan, & Girin et al., 2009).

Losses due to Shattering

Even when harvesting is done mechanically, shattering causes massive losses to Brassica. Grain yield losses in Brassica vary from 10 to 25 percent due to shattering (Price, & Hobson et al., 1996). Insect pest and disease damage also accelerate ripening and pod shattering (Rameeh, 2013) If harvesting is postponed due to poor weather conditions, then shattering can cause 50% yield losses in Canola (Price, & Hobson et al., 1996). If the shedding of seed continues in soil, then it gives rise to weed control problems in upcoming years (Malhi, & Gan et al., 2007). Large seed losses may occur if there is a delay in straight and strong winds occur (Vera, & Downey et al., 2007). It is documented that in cruciferous crops (*B. napus* L, B. juncea L, *B. Rapa* L, Sinapis alba L.) pod shattering resistance is variable (Brown et al., 1997). Moreover, it is described that yield loss for juncea mustard and napus canola: both lose more than 7% of the total seed yield. He also reported that yield losses were lowest for Rapa (2.4%) and juncea (3.8%) canola. When dry weather existed between physiological maturity and straight combining, along with wind movement under the crop canopy, pods broke or split open, resulting in rapid seed loss (Gan, & Yu et al., 2007). (Price, & Hobson et al., 1996) described the losses in winter rape and spring rape. He documented those overall losses in winter rape due to direct cutting are 11% and 4.7 % to 6.4% were from natural shedding. Whereas in the case of spring rape losses are 1.7% to 4.9% for direct cutting and 1.2% to 2.0% for natural shedding.

Shattering in *Brassica napus L.*

Rapeseed is more prone to shattering. The main breeding techniques for developing rapeseed grain yield potential are a good knowledge and application of the morphological, physiological, and genetic basis of grain yield related features in different climatic conditions (Bruce, & Edmeades et al., 2002, & Chauhan, & Singh et al., 2011). Tolerance to shattering is very important for the enhancement of grain yield in rapeseed because crop matures under warm environments and windy summer conditions (Rameeh, 2013). The yield losses in rapeseed are partitioned into two durations, one is before and the other is during harvesting (Chandler, & Corbesier et al., 2005). Progressive ripening (among the plants and between the plants) also influenced the Brassica napus L. (when it is cultivated on a large scale) during harvesting of ripe and immature siliques (Pari, & Assirelli et al., 2012). Even a short period of rapid dehydration can cause a 60% loss of water in siliques and become vulnerable to shattering and causes notable seed losses (Squires, & Gruwel et al., 2003). The weed infestation also increased due to seed losses; this infestation declines the yield of the crop which follows rapeseed in crop rotation. More than 400 kg has-1 or 12% seed losses can be occurred due to pod shattering under unfavorable conditions. (Kosteckas & Marcinkevičiene, 2009). Fully maturation of pods a is more susceptible to splitting which results into the pod shattering and seed losses (Child, & Summers et al., 2003).

How we can reduce shattering:

There is tension in pods of plants which is build-up due to the lignification of the cells that surround the zone of dehiscence at the final stage of pod making and this tension causes pod shattering. (Roberts, & Elliott et al., 2002). Thus, pod shattering can be decreased by decreasing tension in the pod wall or by widening the dehiscence zone. Preharvest seed can be decreased by reducing the maturation time of Brassica napus L (Cavalieri, & Lewis et al., 2014).

Agronomic Management Against shattering in *Brassica napus L.*

The improvement of an ideotype that portrays oilseed rape as progressively resistant to seed misfortune at collect and keeps up elite agronomically relies on the identification of numerous structural characters (Thurling, 1991). These will include the morphological characteristics of the whole plant and raceme, as well as the morphology of individual pods and the interaction of various aspects. As a result of the canopy's character development, a considerable amount of unit shattering occurs inside the crop canopy before harvesting and during harvesting, resulting in pods thumping against one another or stems and branches falling against one another. Other plant characteristics, such as the number of unit points, case length, and breadth, are likely to have a significant impact on mechanical damage (Jonsson & Bengtsson, 1970).

In Pakistan Brassica napus L is cultivated conventionally. Almost 140-160 ha-1 laborers are required for the production of rapeseed in Pakistan. Whereas in other countries (Europe and Canada) 15 labors ha-1 could meet the demand. Rapeseed production is negatively affected due to inept mechanically harvesting procedures and that's why farmers prefer other crops (Wheat, Rice, etc.) in the growing season (ZHANG, & Jun et al., 2012). Delay in harvesting is also a good technique for the estimation of shattering tolerance in rapeseed and making it more tolerant of shattering. Proper land and seedbed preparation also reduced yield losses due to shattering. The seedbed of Rapeseed should be invariable, strong, and moist which enables good seed and soil contact (Tamoor, & Tariq et al., 2018). Equal distribution of the crop residuals, properly organized crop rotations are the requirements of less or minimum tillage, which will create moist, constant, and strong seedbed (Shekhawat, & Rathore et al., 2012). Rapeseed and Mustard have the highest Sulphur requirement among all oilseed crops. One bag of potassium sulfate is enough for good yield and better performance of rapeseed (Mustafa, 2019). The number of irrigations is an important factor for working out of rapeseed. Mostly it needs three irrigations first right after the first month of crop sowing, second at the flowering stage and third is at the seed formation stage. Increased in water amount will increase the water potential of leaves and pods and there will be less shattering. Weeds compete with plants for water, light, and space. Some common weeds for rapeseed are Cleavers, Bassia scoparia, Creeping thistle, Field penny-cress, etc. Proper weed management with herbicides is done to increase the profit and control the shattering. Optimum crop geometry, an equal proportion of NPK fertilizers, mixing of FYM and intercultural operations are good agronomical tools to reduce the shattering in rapeseed. Swathing and direct harvesting are two methods for harvesting of rapeseed (Mustafa, 2019). Use of well-equipped (extended cutter bar table and vertical double knife active dividers) and adjusted combine harvester reels (Špokas & Steponavičius, 2014). The use of good harvest management techniques can also decrease the footprint of unideal meteorological conditions due to pod shattering in rapeseed (Gan, & Malhi et al., 2008).

Genetic Improvements of Brassica. Napus L against Shattering:

Despite the fact that resistance of Brassica napus to shattering is decreased by current breeding methods, certain resistance lines may be found in the diploid progenitors of *B. napus* (B. oleracea and *B. Rapa*), as well as other Brassicaceae members (particularly Brassica juncea, B. carinata, and B. nigra) (MacDonald & Ingram, 1986). The existence of shattering resistant properties in *B. napus* is difficult to establish. Modern technologies, such as embryo rescue, marker-assisted breeding, and novel variation (mutation), on the other hand, may make it much simpler to introduce new rapeseed types into the crop. If we alter the thickness of the pod wall, we may be able to mitigate the detrimental consequences of dehydration (Morgan, & Bruce et al., 1998). The incorporation of such enzymes prevents the dehiscence layer from dissolving and enhances the material's shattering resistance (Jenkins, & Paul et al., 1996).

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