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HERBICIDE TOLERANT PLANT BREEDING IN CEREALS

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Abstract: The breeding of herbicide-tolerant crop plants dates back to the 1980s. To develop herbicide-tolerant varieties, tolerance genes are obtained from naturally occurring gene sources, from material obtained through mutation, or through interspecies gene transfers using biotechnological methods to create genetically modified organisms (GMOs). Most of the imidazolinone-tolerant plants have been improved with conventional plant breeding methods. Imidazolinone herbicides can control weeds, including those closely related to the crop itself and some key parasitic weeds. Imidazolinone-tolerant crop production has benefits of controlling weeds, including those closely related to the crop itself and some key parasitic weeds. There are imidazolinone-tolerant varieties has been improved in corn, rice, canola, sunflower, wheat, barley, lentil, chickpea and sorghum.

Keywords: Cereals, Imidazolinone, Tolerance

INTRODUCTION

Weeds can be controlled either by herbicides or mechanically. Although chemical control is a cheaper and more effective method, it is not always possible to develop selective or broad-spectrum herbicides for every crop. Developing plants resistant to broad-spectrum herbicides to control weeds, and cultivating these crops while using broad-spectrum herbicides, offers advantages to growers. The breeding of herbicide-tolerant crop plants dates back to the 1980s. To develop herbicide-tolerant varieties, tolerance genes are obtained from naturally occurring gene sources, from material obtained through mutation, or through interspecies gene transfers using biotechnological methods to create genetically modified organisms (GMOs). Except for GMOs, the other two methods are classical breeding techniques, and it is easier for plant varieties developed through these methods to enter production. Varieties developed through GMOs are strictly regulated in many countries and are even banned for cultivation in some countries. The cultivation area of herbicide-resistant GMO plants covers 47% of the total transgenic production area worldwide (Prakash et al., 2020). The cultivation areas for herbicide-resistant plants such as corn, sunflower, rice, canola, etc., obtained through classical breeding methods, have vast area.

Broad-spectrum imidazolinone herbicides, which include imazapyr, imazapic, imazethapyr, imazamox, imazamethabenz, and imazaquin, control weeds by inhibiting the enzyme acetohydroxyacid synthase (AHAS), also called acetolactate synthase (ALS).

AHAS is a critical enzyme for the biosynthesis of branched-chain amino acids in plants (Tan et al., 2005). The imidazolinone herbicide group, a broad-spectrum herbicide, controls grasses (jointed goatgrass, feral rye, *Bromus* species, Italian ryegrass, wild oats, and volunteer cereals) and some spring and winter annual broadleaf weeds (shepherd's purse, henbit, field pennycress, chickweed, and mustard species) (Johnson et al., 2023).

Imidazolinone herbicides can control weeds, including those closely related to the crop itself and some key parasitic weeds. A single target-site mutation in the AHAS gene may confer tolerance to AHAS-inhibiting herbicides, making it technically possible to develop the imidazolinone-tolerance trait in many crops (Tan et al., 2005).

Imidazolinone herbicides are very broad spectrum of weeds and low toxicity to animals, birds, fish, invertebrates (Shaner, 2003). Since animals do not synthesize all the amino acids they require and obtain some from plants or bacteria, imidazolinones tend to be less toxic to animals compared to herbicides with other modes of action (Reade and Cobb, 2002). Due to all these characteristics, imidazolinones, belonging to the ALS inhibitor herbicide group, have been prioritized for the development of herbicide-tolerant plants.

Imidazolinone-tolerant Corn

The first imidazolinone-tolerant plant developed is corn. The development of imidazolinone-tolerant corn began in 1982. In tissue culture studies, selection with the imidazolinone herbicide imazaquin from callus resulted in the development of imidazolinone-tolerant corn lines XA17, XI12, QJ22, XS40, ZA54, UV18, AC17, and QT15 from the A188 × B73 hybrid (Shaner et al., 1996; Newhouse et al., 1991). From these mutants, XA17 and XI12 lines were introduced into commercial corn varieties and started being marketed in 1992 as IMI corn. This imidazolinone-tolerant plant production later was called Clearfield® system. Several imidazolinone-tolerant maize lines, including mutant 1 and mutant 2, were also successfully obtained by using the chemical mutagen ethyl methanesulfonate (EMS) to mutagenize pollen from the inbred maize line UE95. Imidazolinone-tolerant maize from this source was subsequently commercialized (Shaner et al., 1996; Bernasconi et al., 1995). Imidazolinone-tolerant corn hybrids are 1000 times more tolerant to imazethapyr and imazapic compared to standard corn hybrids (Newhouse et al., 1990).

The use of IMI-tolerant corn seeds in Clearfield® production in the USA in 2002 was approximately 4.9 million hectares, yet this constituted only 15% of the total corn cultivation area (Tan et al., 2005).

Imidazolinone-tolerant Oilseed rape

Imidazolinone-tolerant oilseed rape was improved with mutation of microspores of the oilseed rape variety Topas with ethyl nitrosourea (Swanson et al., 1989). Five double-haploids survived soil treatment with imazethapyr, and two of the lines showed superior tolerance to imazethapyr. The two most tolerant mutants were P1 and P2, also referred to as PM1 and PM2 (Shaner et al., 1996; Swanson et al., 1989). Later imidazolinone-tolerant oilseed rape varieties were developed with crossing PM1 and PM2 mutants and were first marketed as Smart canola in 1995. Later it was called and marketed as Clearfield® canola (Tan et al., 2005).

Canola Clearfield® cultivation system production area was reached 4-4.9 million hectares, accounting for 20% of the total canola production area in Canada in 2000-2001 (Simard et al., 2002).

Imidazolinone-tolerant Rice

Imidazolinone-tolerant rice was improved with mutation breeding. EMS was used to obtain imidazolinone-tolerant rice rice from seeds, the rice variety AS3510. To select imidazolinone-tolerant rice plant, M_2 plants were sprayed with imazethapyr. A single plant was survived from imazethapyr application (Croughan, 1998). This mutant line was called as 93AS3510, and later, imidazolinone-tolerant rice varieties, CL121 and CL141, were developed with making cross with this imazethapyr tolerant material. Imidazolinone-tolerant rice varieties, CL121 and CL141 first marketed in the USA in 2001 (Croughan, 2003; Gealy et al., 2003). Cypress variety seeds were also used to obtain imidazolinone-tolerant rice. Cypress variety seeds were mutagenized with EMS, later M_2 plants of these seeds were foliar treated with imazapyr or imazapic. Twelve plants survived from the application of imazapyr or imazapic treatment and were confirmed to have tolerance. The seven most tolerant lines from these, were selected for further characterization (Croughan, 2002). Subsequently, two imidazolinone-tolerant rice varieties, CL161 and XL8, were developed from the mutations of this source and first marketed in 2003 (Gealy et al., 2003).

Imidazolinone-tolerant Wheat

Seeds of the French winter wheat variety Fidel were used to improve imidazolinone-tolerant wheat. Sodium azide was used as a mutagen. After mutation M_2 seeds were screened by using a seed treatment with imazethapyr followed by a pre-emergence application of imazethapyr. Four tolerant plants were survived from imazethapyr application, and they were named FS1 (Fidel selection 1), FS2, FS3, and FS4, respectively (Newhouse et al., 1992). Later these four imazethapyr-tolerant lines have been used to improve imidazolinone-tolerant wheat varieties, which were first marketed in 2001. More wheat mutants were also discovered through seed mutagenesis (Pozniak et al., 2004). Seeds of spring wheat variety Teal were mutagenized with EMS, and imazamox was applied to select imidazolinone-tolerant plants in M_2 population. Six lines with moderate to high levels of imazamox tolerance were selected for further genetic study. The lines were designated as TealIMI lines 1A, 9A, 10A, 11A, 15A, and 16A. Two distinctive mutations, different from FS4, were discovered from lines 11A and 15A. Line 15A had the FS4 mutation and another novel mutation. TealIMI 11A possessed a non-allelic mutation to FS4 (Pozniak et al., 2004).

Imidazolinone-tolerant Sunflower (*Helianthus annuus* L.)

An imazethapyr-tolerant wild sunflower population was discovered in a soybean field near Rossville, Kansas, USA (Al-Khatib et al*,* 1998). Seeds of the tolerant wild sunflower population were collected and used to improve imidazolinone-tolerant sunflower as a gene donor (Miller et al., 2000; Miller et al., 2002). IMISUN-1 is the BC_2F_2 seed derived from imidazolinone-tolerant BC_2F_1 plants from the cross HA893/H. annuus, and IMISUN-2 is the BC_2F_2 seed derived from imidazolinone-tolerant BC_2F_1 plants from the cross RHA409//RHA376*2/*H. annuus* (Al-Khatib and Miller, 2000). Later, maintainer line HA425 (BC_2F_6) was improved via pedigree breeding from IMISUN-1, and restorers RHA426 and RHA427 were improved from IMISUN-2 (Miller et al., 2002). The seeds of IMISUN-1 and IMISUN-2 were made available for sunflower breeders to develop imidazolinone-tolerant sunflowers (Al-Khatib and Miller, 2000; Miller et al., 2002). Several commercial seed companies were used these imidazolinone-tolerance trait into their own sunflower lines, and imidazolinone-tolerant sunflower varieties were first commercialized as Clearfield® sunflower in the USA, Argentina, and Turkey in 2003 (Tan et al., 2005).

In Turkey, Clearfield® technology has been used to combat hard-to-control weeds, especially common cocklebur (*Xanthium strumarium* L.) and broomrape (*Orobanche* spp.).

Other Imidazolinone-tolerant Crops

Besides corn, rice, canola, sunflower and wheat; Imidazolinone-tolerant varieties, also have been improved in barley (*Hordeum vulgare* L.), lentil (*Lens culinaris* L.), chickpea (*Cicer arietinum* L.) and sorghum (*Sorghum bicolor* L.) to use this technology for controlling weeds.

Imidazolinone-tolerant varieties are used to control mostly (*Xanthium strumarium* L.) and broomrape (*Orobanche* spp.) in sunflower, red rice in rice, and weed and wild *Brassica* spp. in canola. This technology can also be useful in oat, barley, lentil and chickpea in Turkiye.

MATERIAL AND METHODS

Imidazolinone-tolerant rice, corn, wheat, and barley studies were started after 2000 in Turkey. Introduced imidazolinone-tolerant materials of rice, corn, and wheat and their crosses have been used as material for these studies in Turkey. In addition, mutation studies were conducted to find new imidazolinone-tolerant genes in wheat, barley, and rice. To obtain new imidazolinone-tolerant genes in wheat and barley, the chemical mutagen ethyl methane sulfonate (EMS) was used (seeds were soaked for 8 hours, then placed in water containing 0.4% EMS for 12 hours, washed for 6 hours, then dried and planted). For rice, EMS mutation application with 12 hours of pre-soaking, 6 hours of application at a 0.5% dose, 6 hours of washing, and drying at 38°C for 72 hours is the most suitable method for EMS mutation in rice (Unan et al., 2021).

RESULTS AND DISCUSSION

Studies to Develop Imidazolinone-tolerant Corn Cultivars

Studies to develop imidazolinone-tolerant corn were started in 2013. Local varieties and populations were crossed with introduced imidazolinone-tolerant corn material, and imazamox was applied to segregating material after the F_2 generation. The objective of breeding imidazolinone-tolerant corn is to control weeds, but there are good selective herbicides on the market. For this reason, developing imidazolinone-tolerant corn has slowed down.

Studies to Develop Imidazolinone-tolerant Rice Cultivars

In Turkey, the most successful imidazolinone-tolerant cereal breeding studies were done in rice. Because it is used very successfully to control red rice, a major problem in rice cultivation whose control with other herbicides is not possible. As of the end of 2025, there are 32 registered imidazolinone-tolerant rice varieties, and 12 production permitted rice varieties in Turkey. Imidazolinone-tolerant rice breeding studies hold a significant place in the rice breeding programs of public institutes and private seed companies now.

Imidazolinone-tolerant rice breeding studies will continue; however, in recent years, gene flow between red rice and imidazolinone-tolerant rice has been observed, and imidazolinone-tolerant red rice is seen in many fields. Therefore, in the future, different herbicide groups will be needed to combat red rice. Additionally, imidazolinone group herbicides are insufficient for weed control in rice, especially against barnyard grass. Indeed, new rice varieties resistant to other herbicide groups, such as Provisia, have begun to appear on the market.

Studies to Develop Imidazolinone-tolerant Wheat

The imidazolinone-tolerant wheat variety Buck 55 CL was used in initial crosses with improved wheat varieties well-adapted to the Trakya Region of Türkiye in 2011. Since then, various numbers of crosses have been done to develop imidazolinone-tolerant wheat varieties. Improved wheat varieties, imidazolinone-tolerant segregating material, and selected pure lines are used as plant material in this study.

During imidazolinone-tolerant wheat breeding studies, a modified bulk selection method was used in the segregation population. Selection for imidazolinone-tolerant plants began in the F2 generation and continued through the F3, F4, F5, and F6 generations. To select tolerant plants in the segregating populations, an imidazolinone herbicide containing 40 g L^{-1} of imazamox was applied at a rate of 1250 ml ha^{-1}. For phytotoxicity trials, higher doses of 2500 ml/ha, 3750 ml ha^{-1}, and 5000 ml ha^{-1} of the same herbicide were also applied. In addition to herbicide tolerance, selections were made for resistance to important diseases and for desirable agronomic traits during evaluations in observation nurseries and yield trials.

Yield trial experiments were initiated in the 2017-2018 growing season. Since then, imidazolinone-tolerant bread wheat yield trials have been conducted annually at several locations in the Trakya Region. A Randomized Complete Block Design (RCBD) with four replications was used as an experimental design for all yield trials. There are some imidazolinone-tolerant bread wheat lines ready for registration.

Studies to Develop Imidazolinone-tolerant Barley

To develop imidazolinone-tolerant barley, mutation studies were started in 2021. Imazamox was applied in the F_2 generation, and one imidazolinone-tolerant barley plant was obtained.

To transfer these imidazolinone-tolerant genes to high-yielding barley lines, crossing studies are continued. After crossing, imazamox is applied at the F_2 and later generations.

CONCLUSION

Imidazolinone-tolerant plants offer many advantages. In rice cultivation, they have provided significant benefits in controlling red rice and also reduced weed density. However, due to gene flow from imidazolinone-tolerant rice to red rice in recent years, this technology has lost some effectiveness, creating a need to develop rice resistant to new herbicide groups. In wheat, breeding imidazolinone-tolerant varieties offers great advantages, especially for controlling weed species related to wheat in wheat fields. Another advantage of developing imidazolinone-tolerant wheat varieties is that when wheat is planted after imidazolinone-tolerant previous crop cultivation, if the wheat is not imidazolinone-tolerant, residual herbicide damage can sometimes occur in wheat, especially in dry years. However, this will not be seen if imidazolinone-tolerant wheat is planted. While the same advantage applies to barley and oats, in oats, the control of wild oats with herbicides is not possible; therefore, similar to rice, developing imidazolinone-tolerant oat varieties to control wild oats within oat crops will provide advantages

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