HERBICIDE RESISTANCE BREEDING IN SUNFLOWER, CURRENT SITUATION AND FUTURE DIRECTIONS

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Introduction

Sunflower is one of the most important oil crops in the world due to higher adaptation capability, mechanization use and preference by customers as vegetable oil. Sunflower areas are not larger in the world because the income is lower due to influenced more from environmental conditions as a summer crop. To increase production, it is important for decreasing of effects of factors reduced seed yield utilizing from higher production techniques in addition to develop higher yielding cultivars [43, 79, 47].

Weeds and broomrape *(Orobanche cumana* Wallr.) parasite exist among the most limiting factors for sunflower production in especially in Eastern Europe and Black Sea Region which have more than 60% of world sunflower planted areas [45]. Broomrape is a parasite affecting sunflower yield severely until 100%. Broomprape is holoparasitic weed (angiosperm) which lacks chlorophyll and dependent on host plant for nutrients and it develops new aggressive races historically against sunflower resistant genotypes. New races of broomrape such as F, G and H other than 5 known races (A, B, C, D and E) were observed in mostly in Balkan and Black Sea region and also in Spain [85, 63, 17, 41, 22, 78, 64, 26, 43, 46]. Therefore, new hybrids should have resistant genes against these new races or Clearfield technology [6, 53, 54, 4, 39, 44, 80, 55,18].

Clearfield system with Imidazolinone (IMI) tolerant hybrids and IMI herbicide as post emergence application control successfully broomrape and common weeds [57, 4, 8, 18,]. Similarly, Express Sun system and Sulfonyl Urea (SU) herbicide resistant cultivars and post emergence SU group herbicide is also used efficiently especially in Central and Eastern European countries. Clearfield and Express Sun technologies were accepted widely in almost all sunflower grower countries with restriction of growing of genetically modified crops due to not being biotech product [83, 40, 51, 52, 86, 23, 24, 33, 34, 37, 15, 44, 67, 45, 47].

The development of IMI herbicide resistance in sunflower. Imidazolinone (IMI) tolerant wild sunflower popula¬tion was discovered firstly in soybean field in Kansas, USA in 1996 [1, 59] then IMI tolerance genes were transferred via backrossing and two IMI (IMISUN-1 and IMISUN-2) tolerant lines were developed firstly in USDA sunflower program in Fargo, ND, US [3]. The IMI herbicides control weeds by inhibiting a key enzyme in the branched chain amino acid biosynthetic pathway, acetohydroxyacid synthase (AHAS; EC 4.1.3.18) also known as Acetolactate synthase (ALS) [83, 9-11].

Clearfield system were introduced to farmers firstly in 2003 in Turkey lately Argentina, USA and other countries [17]. In Clearfield technology, IMI post emergence herbicides (Imazamox (40 g/l)) applied 6-8 leaf stage control efficiently both broomrape and major broadleaf weeds such as *Xanthium strumarium* Wallr. *Chenopodium album* L., *Echinochloa cruss-galli*, *Sinapsis arvensis* L., *Amaranthus* spp., *Solanum nigrum* L., *Datura stramonium* L. ragweed, *Avena* spp. etc. resulting important yield losses in sunflower [16, 54, 65, 42, 18, 51, 36, 52, 49, 27, 21, 15, 44]. After application IMI herbicide, chlorosis could seems depends on applied herbicide amount and application method but it disappear generally in a week [23, 62]. On the other hand, Anastasov H. (2010) indicated that imazamox results considerable changes in the sunflower leaf anatomy, a reduction of stomata number and an increase in the thickness of leaf lamina (blade) after applied at suggested dose as post emergence.

Ahas locus confers resistance to IMI tolerant sun¬flower [12, 50]. The inheritance of IMISUN is additively controlled by two genes, one partially dominant allele *Ahasl1-1* and a modifier gene [59, 12, 38]. However, this IMI trait has lower oil content in the seed due to the wild parent around the resistant gene [74-76].

The second IMI tolerance source known as CL Plus was developed by seed mutagenesis and selection with imazapyr in sunflower [72]. While the Clearfield® system is based on two genes (*Ahasl1-1* and an enhancer, [83]), the CL Plus system, based on the allele *Ahasl1-3* alone or in combination with Ahasl1-1 [71-76]. This new traits present better stability of the herbicide tolerance in different environmental conditions, permit developing new herbicide formulations providing more flexible and reliable weed control, higher oil content, etc. than previous IMISUN trait [71-73, 88, 89, 68, 69]. Clearfield Plus® trait results higher accumulation of biomass after IMI application at the above-ground and root level because of displaying lower inhibition of the AHAS enzyme extracting by IMI [73, 88, 89, 84].

The development of SU herbicide resistance in sunflower. Sulfonyl Urea (SU) herbicide tolerance sunflower were discovered from wild sunflower isolates ANN-KAN and ANN-PUR in Kansas, US, (which is the same field discovered IMI resistance wild population) [2]. SURES-1 and SURES-2 lines were developed with resistance to sulfonylurea herbicides by introgression of mutations, respectively then transferred into elite breeding lines produced from these crossings [58, 25, 60, 31, 32]. The target-site-tolerance is the result of the mutation P197L at the *Ahasl1* locus and the inheritance of this trait is dominant way as exhibiting completely resistance to tribenuron [60, 50, 19, 28, 29, 74-76]. While White *et al*. (2003) mentioned that at least two ALS gene copies existing in these SU sunflower lines, Bruniard and Miller (2001) pointed out three putative ALS genes. However, Miller and Zollinger (2004) indicated that differences in crop injury among SURES lines (*Ahasl-2/Ahasl1-2*) are the result of the presence of modifier genes. Some studies were carried out to determine SU resistance allele specific markers and in vitro techniques in the lab [13, 20].

ExpressSun® technology is the same type of tolerance as SURES obtained by EMS mutagenesis over the line HA89 [82]. Sulfo¬nylurea tolerant sunflower cultivars (ExpressSun technology) were introduced for farmers in 2007 (USA) and using commonly in many countries especially in Eastern Europe [77, 30, 28, 29, 56].

SU herbicides control more weeds and also cheaper than IMI are used widely in sunflower production in the world. However, SU resistant hybrids have the less control on both broomrape and some common weeds such as Xanthium, Cirsium, etc. so they should be combined with broomrape resistance together [23].

Current stiuation herbicide resistance in sunflower. Farmers like this technology due to offering well control on both broomprape and also common weeds but they should wait until 6-8 leaves stage to apply IMI herbicide for efficient broomrape control. These delaying applications result sometimes not well control of already grown weeds. Therefore, combining broomrape resistant genes with IMI resistance in the same hybrid give farmers more options both for application time and amount depending on weed infestation in their fields [41, 44]. Additionally, seed companies also develop new tolerant hybrids every years mostly combining or adding new traits to broomrape tolerance such as downy mildew resistance as well as IMI herbicide resistance together because Clearfield system is one of the best and efficient option to control both broomrape and major broadleaf weeds [23]. Now, sunflower hybrids combined these traits (IMI + Orb, Orb + SU) have started to sell recently and are preferred widely by sunflower growers. However, due to CL Plus and ExpressSun resistant genes developed by chemical mutation it needs provisional contracts to use by sunflower breeders widely [44].

Although Clearfield and SU technologies were used widely, there are some arising problems in the production such as herbicide residue problems and effects on following crops, gene escaping to wild species, weed tolerance, tolerant sunflower cultivars response to ALS inhibiting her¬bicides, volunteer plants of tolerant sunflower cultivars have lower sensitivity to other ALS inhibiting herbicides compared to conventional cultivars and hard control of these volunteer sunflower plants [40, 71, 74, 87, 81, 7, 66, 70, 44, 45, 33-35].

Future directions of herbicide resistance in sunflower. Furthermore, broomrape and herbicide resistant hybrids combined all three traits (Orb + IMI + SU) will be developed with using IMI and SU resistant genetic material soon in the future. These new hybrids combined these traits present more economical results to sunflower producers as reducing cost and increasing income per area with giving herbicide selection based on broomrape and weeds in their fields [44].

To provide sustainable and durable broomrape management, herbicide tolerance should be incorporated with resistant genes to different broomrape races in order to avoid breaking of resistance rapidly in the following years. New, more reliable, lower cost and rapid screening methods should be added for efficient herbicide tolerance in addition to phenotypic control and tests at V2-V4 stages such as molecular markers, in vitro screening, etc. Some proved methods were also developed such immature embryo [9], seed germination bioassays for screening IMI-tolerance [84, 10, 27] and SU-tolerance [20] and marker assisted selection especially for introgression of genes for herbicide resistance into high yielding sunflower germplasm [50, 13, 71-76]. On the other hand, new herbicide molecules need to develop for efficient weed control in sunflower due to limited selective herbicides for the sunflower and higher cost of herbicide registration [14, 74-76]. Therefore, research studies should be performed to develop new herbicide resistance genes to supply alternative choices, to increase the productivity and the competitive ability in sunflower.

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