RESISTANCE MECHANISMS OF ALUMINUM (Al^{3+}) PHYTOTOXICITY IN CEREALS: PHYSIOLOGICAL, GENETIC AND MOLECULAR BASES

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Mecanismos de resistencia a la fitotoxicidad por aluminio (Al^{+3}) en cereales: bases fisiológicas, genéticas y moleculares

Keywords: Aluminum tolerance, cereals, organics acid, *ALMT1* gene, triticeae family.

ABSTRACT

Aluminum (Al) toxicity is one of the main factors limiting crop productivity in acid soils around the world. In cereals, this problem can affect between 30 and 40% of crop yields. One way to reduce the toxic effect of Al is to neutralize the acidity with calcareous amendments. However, this practice is demanding and not very effective. An alternative is the search for genetic variability in the genome of cropping grasses and/or their wild relatives to resist Al. The development of biotechnology and molecular genetics approach has facilitated the understanding of the physiological, genetic and molecular bases in the process of ameliorating these species. This review presents the main physiological mechanisms of Al resistance and the genetic and molecular bases that explain the degree of resistance between different cereals species.

Palabras Claves: Tolerancia aluminio, cereales, ácidos organicos, gen *ALMT1*, familia Triticeae

RESUMEN

La toxicidad por aluminio (Al), es uno de los principales factores que limitan la productividad de los cultivos en los suelos ácidos alrededor del mundo. En cereales este problema puede afectar entre 30 a 40% los rendimientos de los cultivos. Una de las opciones para reducir el efecto tóxico del Al, es neutralizar la acidez con el uso de enmiendas calcáreas. Sin embargo, esta es una práctica muy laboriosa y poco efectiva. Una alternativa es la búsqueda de variabilidad genética para la resistencia a Al en el genoma de gramíneas cultivadas y/o sus parientes silvestres. El desarrollo de la biotecnología y la genética molecular han

facilitado el entendimiento de las bases fisiológicas, genéticas y moleculares en el proceso de mejoramiento de estas especies. En este review se presentan los principales mecanismos fisiológicos de la resistencia a la fitotoxicidad por Al y los fundamentos genéticos y moleculares que explican el grado de resistencia entre las diferentes especies de cereales.

INTRODUCTION

Aluminum (Al) phytotoxicity is one of the major agronomic problems in acid soils. The concentration of Al ions in soil solution is high unless the pH<5.0 (Kochian, 1995; Schmohl and Horts, 2002; Zhang *et al*., 2007), compromising more than 40% of the potentially arable soils in the world (Foy *et al*., 1978; Kochian, 1995; Zheng *et al*., 1998; Delhaize *et al*., 2004). This problem is exacerbated by the use of ammonium fertilizers and acid rain (von Uexkull and Murtert, 1995). The main symptom of Al toxicity is a rapid inhibition of root growth, which may translate to a reduction in vigor and crop yields (Rengel, 1992; Kochian *et al*., 2005). Plants have different mechanisms to resist or tolerate the toxic effect of Al in response to this stress. These resistance mechanisms in plants have been classified as a) external or exclusion through the exudation of organic acids from the radical apexes and subsequent chelation of the root in the rhizosphere and b) internal or Al-tolerant since Al chelation is produced inside the cell and then later stored and compartmentalized in organelles like the vacuole (Kochian, 1995; Ramgareeb *et al*., 2004). Several investigations have reported that Al interferes with the cell division of radical apexes, increases rigidification of the cell wall by crossing with pectins, reduces DNA replication by increasing the rigidification of the double helix (Rout, 2001), interferes with the signal transduction pathways, alters cytoplasmic Ca^{2+} levels (Jones *et al*., 1998) and inhibits phospholipase C (PLC) activity of the phosphoinositide pathway associated with Ca^{2+} signaling (Jones and Kochian, 1995; Ramos-Díaz *et al*., 2007).

The genetic bases for Al resistance have been studied in a limited number of species, such as wheat, oats, rye, triticale, sorghum (Delhaize *et al*,. 1993b; Fontecha *et al*., 2007; Nava *et al*., 2006; Budzianowski *et al*., 2004; Caniato *et al*., 2007). Although the resistance seems to be a multigenic trait in most of the plants studied, in some species it seems to be codified by a simple dominant locus (Jones and Ryan, 2003). In species like wheat, barley and rye, loci for Al resistance have been mapped on the long arm of chromosomes 4D, 4H and 4R, respectively (Luo *et al*., 1996; Tang *et al*., 2000; Miftahudin *et al*., 2002; Miftahudin *et al*., 2005), suggesting that this position may be conserved for Al resistance in cereals.

Current knowledge of the molecular physiology behind Al tolerance together with the genetics that control this trait make it possible to project significant advances in the production of tolerant varieties in various species of sensitive cereals. Nevertheless, as the complexity of genetic control seems to vary between species – *Oryza sativa*, for example, presents a polygenic control for Al tolerance (Nguyen *et al*., 2003) – the amelioration process also becomes more complex. Although conventional amelioration methods have proven useful in identifying tolerant varieties in several crops (Riede and Andeson, 1996; Gallego and Benito, 1997; Tang *et al*., 2000), these alone do not guarantee an efficient gene transference process to other elite materials. It is possible, however, to increase the efficiency of conventional amelioration by combining it with biotechnological strategies to reduce costs and selection time.

To do this, one must understand the physiological, genetic and molecular bases that govern these mechanisms. Hence, this review aims to present the main physiological mechanisms of Al phytotoxicity resistance and the genetic and molecular bases that explain the degree of resistance among the different cereal species.

Aluminum (Al^{3+}) Toxicity, symptoms in plants

Al mainly affects plants by inhibiting radical growth (see Figure 1). This can be seen in the primary and lateral root apexes, which also become thick and turn brownish-gray (Kinraide, 1988; Roy *et al*., 1988; Rout *et al*., 2001). These symptoms become evident after a few minutes or hours of the plants being exposed to micromolar concentrations of Al in hydroponic solutions (Ryan *et al*., 1993; Blancaflor *et al*., 1998; Sivaguru and Horst, 1998; Zhang *et al*., 1998; Vazquez et al., 1999; Ma *et al*., 2002; Rengel and Zhang, 2003). Radical inhibition coincides with a decline in cell division (Wallace and Anderson, 1984; Horst, 1995; Frantzios *et al*., 2001) and elongation of the root cells, which then induces significant rigidification of the cell wall by crossing with pectins (Rout *et al*., 2001; Jones *et al*., 2006). This alteration prevents the water absorption essential to the transport of nutrients through the apoplast, eventually causing a decrease in yield and grain quality (Zheng and Yang, 2005; Raman *et al*., 2002). Furthermore, Al also triggers membrane lipid peroxidation and apoptosis or programmed cell death (PCD) (Pan *et al*., 2001; Barceló and Poschenrieder, 2002). It has been reported that prolonged exposure to this element can induce and produce responses of rapid change in other biochemical and physiological processes (Rengel and Zhang, 2003). This is why the symptoms at foliar level resemble phosphorus deficiency, preventing plant growth, turning mature leaves dark green, stems purple and killing leaf apexes (Wang *et al*., 2006). In other cases, Al toxicity reduces calcium (Ca) transport, making young leaves curl, preventing the development and growth of the petiole (Rout *et al*., 2001). It has also been described that Ca is removed by Al from the apoplast, but it is highly unlikely that this is the cause of Al toxicity in wheat given that radical growth could be inhibited in nutrient solutions with low concentrations of Al without removing the Ca from the apoplast (Ryan *et al*., 1997). Excesses of Al also induce symptoms of a Fe deficiency, which was observed in *Sorghum bicolor* (Clark, 1981), *Triticum aestivum* (Foy and Fleming, 1982) and *Oryza sativa* (Rout *et al*., 2001). Several studies indicate that Al affects the normal operation of cell membranes, causing enzymatic disorders and affecting the nuclear DNA (Maustakas *et al*., 1992; Matsumoto *et al*., 1997; Sasaki *et al*., 1997). It acts on the phosphate groups, altering their topology and recognition by polymerase DNA, modifying the entire functioning of the replicative machinery due to the increased rigidity of the double helix (Rout *et al*., 2001; Mossor-Pietraszewska, 2001; Zhang *et al*., 2002). In addition, Al is closely linked to other DNA-associated molecules, such as phosphorylated proteins (histones) (Kochian, 1995). Al interferes in the normal operation of the Golgi apparatus and in the peripheral cells of the apex of intact roots, in their quiescent center, mitotic activity and DNA synthesis (Rout *et al*., 2001). Al may also affect the mechanism that controls the organization of cytoskeletal microtubules as well as the polymerization of tubulin by delaying disassembly during mitosis (Franzios *et al*., 2000). This would affect the direction of the microtubules, which is closely related to cell expansion (Zheng and Yang, 2005).

Aluminum (Al^{3+}) tolerance mechanisms

Species can vary in their ability to grow in acid soils with severe Al phytotoxicity (Jones and Ryan, 2003). Al tolerance

mechanisms have been classified into two main types: a) those that exclude Al from the root cells and b) those that allow Al to be tolerated once it has entered the plant cells (Barceló and Poschenrieder, 2002). Species in tropical areas are very resistant to Al stress and some of these species can accumulate high concentrations of Al in the leaves, greater than 1% of their dry weight (Jones and Ryan, 2003). By contrast, cereals like *Secale cereale*, *Zea mays*, *Hordeum vulgare*, *Triticum aestivum*, X *Triticosecale*, *Sorghum bicolor* and *Avena sativa* do not accumulate high concentrations of Al internally but rather use the Al exclusion mechanism through organic acid exudation (Li *et al*., 2000; Piñeros *et al*., 2002; Ma *et al*., 2004; Delhaize *et al*., 1993b; Magalhaes *et al*., 2007; Caniato *et al*., 2007; Nava *et al*., 2006). This would be one of the most widely used mechanisms by most of the species studied.

External tolerance mechanism (exclusion)

Some species detoxify Al in the rhizosphere by exuding organic acid from their roots (Miyasaka *et al*., 1989; Li *et al*., 2002). This exudation is located in the radical apexes of some species (see Table 1), as this is a region which is very sensitive to Al toxicity due to constant cell division and elongation (Mossor-Pietraszewska, 2001). The organic acids commonly secreted are malate, citrate and oxalate. Malate and citrate are present in all cells given that they are involved in the mitochondrial respiratory cycle (Jones and Ryan, 2003).

Organic acid levels vary between species, cultivars and even between tissues of the same plant under identical growth conditions. In addition, organic acid biosynthesis and accumulation increase drastically in response to environmental stress (López-Bucio *et al*., 2000). It has been observed that tolerant genotypes exude a greater amount of organic acids that sensitive genotypes, which would support the notion that organic acid exudation is an Al tolerance mechanism (Delhaize *et al*., 1995). However, it has been reported that Al-sensitive species of wheat show a greater accumulation in the cortical tissue (5 to 10 times more) than the tolerant genotypes exposed for the same period of time (Delhaize *et al*., 1993a).

Some organic acids such as citrate, malate and oxalate are able to form stable complexes with Al (Ma *et al*., 2001; Jones and Ryan, 2003; Guo *et al*., 2007), where the Al-citrate complex bond is strongest, followed by the Al-oxalate and Al-malate complexes, which are insoluble and not available for plants (Jones and Ryan, 2003). This is because Al is a metal that tends to form strong complexes with the oxygen donor ligand (Barcelo and Poschenrieder, 2002). The transport of these organic acids from the radical cells is mediated by the anionic channel activity in the plasma membrane (Ma *et al*., 2001). These anionic channels might be Al-activated, which was demonstrated using the patch-clamp technique on isolated protoplasts of wheat and maize radical apexes (Ryan and Jones, 2003). Using anionic channel inhibitors such as niflumic acid would support the existence of these channels as elements for organic acid exudation in response to Al (Ryan *et al*., 1997; Kollmeier *et al*., 2001; Piñeros and Kochian, 2001).

Conversely, it has been observed that Al also induces the exudation of certain phenolic compounds, such as catequin and quercitin, from maize radical apexes, forming stable complexes with these compounds; it is likely that they can contribute to Al tolerance, but more research is required to confirm this hypothesis (Jones and Ryan, 2003).

Internal tolerance mechanism (inclusion)

Another proposed detoxification mechanism is internal tolerance or inclusion (Taylor, 1991; Kochian, 1995; Zheng *et al*., 1998). Once the Al enters the cell, the Al^{3+} cation concentration free in the cytoplasm will be very low due to the high pH of the cytoplasm

(pH 7.0). However, it has been indicated that these internal Al concentrations can be dangerous (Jones *et al*., 1998). Al also exhibits a high affinity for the oxygen ligand, which allows it to compete with other ions for metabolically important sites, despite a large difference in their concentrations (Martin, 1986; Ma *et al*., 2001; Jones and Ryan, 2003). It has been observed that the Al-ATP bond is less than the bond of Al-citrate or Al-oxalate complexes. This may indicate that organic anions are able to protect plants by Al chelation in the cytosol. The metallic anion complex could then be transported around the plant for its storage (Jones and Ryan, 2003). This mechanism immobilizes, compartmentalizes or detoxifies the Al from the simplast (Zheng *et al*., 1998; Guo *et al*., 2007). The formation of less toxic Al complexes seems to be a prerequisite for tolerating the high concentrations of internal Al that have been observed in such plants as *Hydrangea macrophylla*, *Fagopyrum esculentum* and *Melastoma malabathricum*, able to accumulate high concentrations of Al (Zheng *et al*., 1998; Barceló and Poschenrieder, 2002; Jones and Ryan, 2003). In *Fagopyrum sculentum*, the organic anions chelate the Al in different tissues, as in the radical cells and the vacuole of the cells of the leaves (Zheng *et al*., 1998; Jones and Ryan, 2003). Moreover, Al-accumulating plants have been identified; these hyperaccumulators accumulate more than 1000 mg kg^{-1} of Al in the leaves (Jansen *et al*., 2002). It has been reported that a high Al accumulation in stems involves the transport of Al-soluble complexes through the xylem and the subsequent innocuous accumulation, solid or soluble, in the vacuole of the leaves or in the apoplast. The unstained Al in the leaves of various accumulators suggests that Al can be transported to the phloem (Barceló and Poschenrieder, 2002).

Genetic bases for Al^{3+} tolerance in cereals

Genetic control for Al tolerance has been studied on a limited number of species of agricultural importance. In cereals like wheat, barley, rye, sorghum and oats this trait seems to be codified by only one major gene (Tang *et al*., 2002; Raman *et al*., 2002; Gallego and Benito 1998; Miftahudin *et al*., 2002; Magalhaes *et al*., 2004; Nava *et al*., 2006). In wheat, the major locus that conditions Al tolerance (Alt_{BH} gene) has been mapped in segregate populations, linked to some molecular markers and localized on the long arm of the chromosome 4D; this locus might control nearly 85% of the phenotypical variation of the trait (Riede and Anderson, 1996; Luo and Derorak, 1996; Rodríguez-Milla and Gustafson, 2001; Raman *et al*., 2005; Raman *et a*l., 2008). Yet in such species as barley and rye the loci for Al tolerance has also been identified on the long arm of chromosomes 4H and 4R (*Alp* and *Alt3* genes), respectively (Tang *et al*., 2000; Miftahudin *et al*., 2002; Miftahudin *et al*., 2005). The position reserved for these Al tolerance loci suggests that this trait in the tribe Triticea (wheat, barley and rye) is controlled by mutations in orthologous loci (Magalhaes, 2006). The locus for Al tolerance (Alt_{SB} gene) in sorghum was recently mapped in the terminal region of chromosome 3 (Magalhaes *et al*., 2007). This major gene is responsible for 80% of the phenotypic variation for Al tolerance in mapped sorghum populations (Magalhaes *et al*., 2007). Other investigations, however, have detected polygenic inheritance for Al tolerance in the Atlas 66 wheat cultivar and not all the genes were localized on the chromosomes of genome D (Berzonsky, 1992). Raman *et al*. (2005) identified a major quantitative trait locus (QTL) for Al tolerance in Atlas 66, localized on

chromosome 4DL. Zhou *et al*. (2001) identified a smaller QTL in this same cultivar localized on chromosome 3BL. Therefore, but to a lesser extent, Al tolerance also seems to be regulated by polygenic traits.

Molecular bases for Al^{3+} tolerance in cereals

The molecular bases for Al tolerance have made it possible to identify and recently to clone a malate transporter that is codified by major gene *ALMT1* (aluminum-activated malate transporter) in isogenic lines of wheat, which is constitutively expressed in the root apexes, with higher levels of malate exudation being observed in the roots of the ET8 lines (Al-tolerant) than in the ES8 lines (Al-sensitive) (Sasaki *et al*., 2004; Hoekenga *et al*., 2006). This gene is a member of a new family of membrane proteins and corresponds to the major Al tolerance locus Alt_{BH} (Raman *et al*., 2005). The location of this malate transporter in the plasma membrane of the radical apexes was confirmed through expression analysis using green fluorescent protein (GFP) on onion and tobacco cells (Yamaguchi *et al*., 2005). Moreover, the heterologous expression of *ALTM1* conferred Al resistance on barley plants by dramatically increasing the malate exudation associated with the increase in Al tolerance in hydroponic crops and acid soils, whereas in rice the expression of *ALMT1* significantly increased the flow of Al-activated malate, but not Al tolerance (Delhaize *et al*., 2004). This is attributed to the insufficient amount of malate released to confer Al resistance on rice (Kukui *et al*., 2007). Another aspect to consider is the role of malate, since compared to oxalate or citrate, this organic acid is the one that exhibits the least capacity for chelating Al ions (Ma *et al*., 1998; Ma *et al*., 2001). *ALMT1* co-segregation Al tolerance in separate F_2 and F_3 populations derived from crossing the isogenic lines (Sasaki *et al*., 2004; Zhou *et al*., 2007). Al-induced gene expression studies have identified genes that

Figure 1: Phenotypic expression of cultivar Al-tolerant, and Al-sensitive of wheat, in hydroponic solution at 20 µM $AlCl_3$. Inhibition of the radical growth is observed in the Al sensitive cultivar (Inostroza-Blancheteau et al. 2005).

Figura 1: Expresin fenotípica en un cultivar de trigo tolerante y uno sensible a Al, en solución hidropónica a 20 µM $AlCl_3$. Se observa inhibición del crecimiento radical en el cultivar sensible (Inostroza-Blancheteau et al. 2005).

are expressed differentially between two isogenic lines of NILs wheat (Chisholm-T, tolerant and Chisholm-S, sensitive), using suppression subtractive hybridization (SSH) and microarray, where 57 genes were expressed differentially during the first exposure to Al. Among these, 28 genes including *ALMT1*, ent-kaurenoic, ß-glucosidase, lectin, histidine kinase and phospoenolpyruvate carboxylase exhibited abundant transcripts in Chisholm-T, facilitating Al tolerance. These results suggest that Al tolerance may be co-regulated by multiple genes with various functions (Guo *et al*., 2007). Then again, studies of functional genomics have determined the structure and chromosomal location of *ALMT1* by physical mapping on chromosome 4DL, suppressing ditelosomic lines (Chinese Spring), which coincides with the loss of Al tolerance. The structure that codifies this gene is pb 1388, with six exons interrupted by five introns (Raman *et al*., 2005), of which two alleles for this gene are reported (ALMT1-1 allele for Al tolerance and ALMT1-2 allele for Al sensitivity). The introgression of these loci in the genetic background of wheat may be an option for developing tolerant wheat cultivars for acid soils with high levels of available Al (Stodart e*t al*., 2007).

In barley, mapping with AFLP and RFLP markers has localized the *Alt* gene, which is responsible for Al tolerance. RFLP analysis localized the gene on the long arm of chromosome 4H (4HL), at 2.1 cM proximal from the marker *Xbcd1117* to 2.1 cM distal from the markers *Xwg464* and *Xcdo1395* (Tang *et al*., 2000; Raman *et al*., 2002). In species like barley, highly saturated genetic maps have been used where locus *Alp* could be identified, delimited at 0.2 cM by the markers *ABG715* and *HvGABP* and using double haploid lines of tolerant (Dayton) and sensitive (Zhepi 2) cultivars and segregant populations F_2. Wang *et al.* (2007) identified a candidate gene *HvMATE* (*Hordeum vulgare* Multidrug and Toxic Compound Extrusion). This gene may control Al tolerance in barley. Recently, through mapping analysis and microarray, the gene was identified (*HvAACT1*), responsible for citrate exudation, activated by Al, using an Al- tolerant cultivar (Murasakimochi) and an Al-sensitive cultivar (Morex), there being a correlation between the expression of the *HvAACT1* gene and citrate exudation, evaluated in barley cultivars with different degrees of Al tolerance (Furukawa *et al*., 2007). This demonstrated that *HvAACT1* is an Al-activated citrate transporter responsible for tolerance in barley. While, in other studies conducted on rye, four different genes for Al tolerance have been identified (*Alt1, Alt2, Alt3* and *Alt4*) localized on chromosome 6RS, 3RS, 4RL and 7RS, respectively (Ma *et al*., 2000; Aniol, 2004; Fontecha *et al*., 2007, Matos *et al*., 2007), this being the cereal species most tolerant of Al toxicity after rice, wheat and barley (Xue *et al*., 2007).

Triticale (X *Triticosecale* Wittmack), a crossbreed of wheat and rye, contains a complete genome from the rye chromosomes (AABBRR), which permits adaptation to marginal atmospheres with a good yield potential, presenting high levels of Al tolerance, approaching those of wheat and rye (Kim *et al*., 2001). Evaluations on winter (cv. Presto) and spring (Rhino) hexaploid triticale lines with D-genome disomic substitution of the wheat chromosome were analyzed, where lines 6 and 9, respectively, show a relative increase in Al tolerance over what was observed in the control lines (Budzianowski and Wos, 2004).

In rice (*Oryza sativa* L.), some quantitative trait loci (QTL) have been analyzed for Al tolerance based on radical growth rate (RGR) using linkage maps and recombinant inbred lines (RILs). These lines were derived from crossing the Al-tolerant Asominori cultivar with the Al-sensitive IR24 cultivar, and 3 QTLs (qRRE-1, qRRE-9 and qRRE-11) were detected on chromosomes 1, 9 and 11, respectively, with a phenotypical variance from 13.5 to 17.7% (Xue *et al*., 2006; Xue

Table 1: Influence of Al on organic acid release from roots of different species of the Triticeae family. This table shows the main organic acids exudated from radical apexes or whole root (Adapted from Barceló et al. 2000; Jones and Ryan, 2003).

Tabla 1: Influencia del Al sobre la liberación de ácidos orgánicos desde las raíces de diferentes especies de la familia Triticeae. Esta tabla muestra los principales ácidos orgánicos exudados desde el ápice radical o la raíz entera (Adapted from Barceló et al. 2000; Jones and Ryan, 2003).

Plant species	Organic acid	Tissue measured	References
Wheat (*Triticum aestivum* L.)	Malate	Root apexes	Ryan *et al.*, 1995
Barley (*Hordeum vulgare* L.)	Citrate	Root apexes	Gallardo *et al.*, 1999
Rye (*Secale cereale* L.)	Citrate, malate	Whole root	Li *et al.*, 2000
Oats (*Avena sativa* L.)	Citrate	Whole root	Zheng *et al.*, 1998
Rice (*Oryza sativa* L.)	Citrate	Whole root	Ishikawa *et al.*, 2000
Triticale (X *Triticosecale* W.)	Citrate, malate	Whole root	Ma *et al.*, 2000
Sorghum (*Sorghum bicolor* L.)	Citrate	Whole root	Magalhaes *et al.*, 2007

et al., 2007). The alleles of the Asominori cultivar of the three QTLs were associated with the increase in Al tolerance. qRRE-9 is expressed in the genetic backgrounds of both IR24 and Asominori/IR24 (Xue *et al.*, 2007). In addition, in two tolerant sorghum cultivars, a major locus (Alt_{SB}) for Al tolerance has been found (Magalhaes *et al.*, 2004). This locus might be associated with Al tolerance through citrate exudation from the radical apexes. Most recently through of positional cloning was identified a gene encoding a member of the multidrug and toxic compound extrusion (MATE) family, an aluminum-activated citrate transporter, responsible for tolerance in sorghum (Magalhaes *et al.*, 2007).

CONCLUSIONS

Cereals are the world's primary nutritional source. They constitute a basic food for humanity and are an important source of vitamins and energy. They are also an important pool of genetic resources that can be used to improve the species. Most of these species are cultivated in acid soils, with high concentrations of phytotoxic Al (Al^{3+}), reducing grain yield and quality. Al tolerance in cereals might be increased by incorporating currently tolerant genes into the genetic pool of the Triticeae family. The search for genetic variability in crop species and/or its wild relatives is crucial for this purpose. Currently, advances in biotechnology and molecular biology have enable to discover and understand the physiological, genetic and molecular mechanisms present in these species. The advances have allowed the use of suitable tools and appropriate methodologies for effective improvement and reducing the selection time and costs.

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