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Durum wheat improvement against fungal pathogens by using protein inhibitors of cell wall degrading enzymes

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Abstract. We report the use of three glycosidase inhibitors, the bean polygalacturonase inhibiting protein 2 (PvPGIP2), the kiwi pectin methyl esterase inhibitor (AcPMEI), and the *Triticum aestivum* xylanase inhibitor III (TAXI-III), to control leaf blotch and Fusarium Head Blight (FHB) symptoms caused by the fungal pathogens *Bipolaris sorokiniana* and *Fusarium graminearum*. We produced transgenic durum wheat lines by particle bombardment using these inhibitors singly or in combination. We pyramided these transgenes also by classical crossing of transgenic lines carrying a single transgene. Phyto-pathological tests performed in controlled conditions showed that the expression of these inhibitors has the potential to engineer a broad-spectrum disease resistance in wheat.

Keywords. Disease resistance – Cell wall degrading enzymes – Glycosidase inh bitors – Fungal pathogens – *Triticum durum.*

Amélioration du blé dur contre les pathogènes fongiques à l'aide de protéines inhibitrices des enzymes dégradant la paroi cellulaire

Résumé. *Nous allons nous intéresser à l'utilisation de trois inhibiteurs de glycosidases, la protéine 2 inhibitrice de la polygalacturonase des légumineuses (PvPGIP2), la protéine inhibitrice de la pectine méthylestérase (AcPMEI) du kiwi, et la protéine inhibitrice de xylanases III de Triticum aestivum (TAXI-III), pour contrôler les symptômes de la tache foliaire et de la fusariose de l'épi (FHB) causés par les pathogènes fongiques Bipolaris sorokiniana et Fusarium graminearum. Nous avons produit des lignées de blé dur transgéniques par bombardement de particules en utilisant ces inhibiteurs seuls ou en combinaison. Nous avons aussi pyramidé ces transgènes par croisement classique des lignées transgéniques portant un transgène unique. Les tests phyto-pathologiques réalisés en conditions contrôlées ont montré que l'expression de ces inhibiteurs permet d'élaborer une résistance aux maladies à large spectre chez le blé.*

Mots-clés. *Résistance aux maladies – Enzymes dégradant la paroi cellulaire – Inhibiteurs de glycosidases – Pathogènes fongiques – Triticum durum.*

I – Introduction

Broad-spectrum and durable resistance to diseases is one of the most attracting perspective in breeding projects aimed at increasing crop resistance. Since most microbial pathogens need to surmount the plant cell wall to penetrate the host tissue, the reinforcement of this complex compartment should increase the capacity of the host plant to resist the attack of different pathogens. We pursued this goal by enhancing the host ability to abolish or limit the activity of Cell Wall Degrading Enzymes (CWDEs) secreted by pathogens during the penetration and colonization of the host tissue (Ten Have *et al*., 2002).

Plants counteract CWDEs by expressing protein inhibitors which contrast the activity of these degradative enzymes (Juge *et al*. 2006). These inhibitors include polygalacturonase inhibiting

protein (PGIP), xylanase inhibitor (XI), pectin lyase inhibiting protein (PNLIP), xyloglucan-specific endoglucanase inhibitor protein (XEGIP) and pectin methyl esterase inhibitor (PMEI).

We concentrated our efforts on the containment of the activity of two different CWDEs: the polygalacturonases (PGs) and the xylanases.

PGs are among the first CWDEs secreted by fungal pathogens during infection and in some patho-systems they are virulence factors (Ten Have *et al*., 2002). PGs depolymerize the cell wall pectin, a minor component of wheat cell wall, and are inhibited by PGIPs (De Lorenzo *et al*., 2001). PG activity is also negatively affected by a high degree of pectin methyl esterification (Bonnin *et al*., 2002). The level of pectin methyl esterification is controlled by the activity of plant pectin methyl-esterases (PMEs), which remove the methyl groups, and by its protein inhibitor PMEI (Wolf *et al*., 2009). Thus, PMEI may indirectly affect negatively the activity of PGs by maintaining a high degree of pectin methyl esterification.

Xylanases are key enzymes in the degradation of xylans, a main component of wheat cell wall (Vogel, 2008). These enzymes have been shown to be virulence factors for the fungal pathogen *Botrytis cinerea* (Brito *et al*., 2006). The activity of microbial xylanases is controlled *in vitro* by XIs (Dornez *et al*., 2010).

II – Observations

By using a transgenic approach we showed that the constitutive expression of PGIP or PMEI endows durum wheat with new capacities to control the activity of fungal PGs, possibly through a direct interaction or indirectly by modifying the level and pattern of methyl esterification of cell wall pectin (Janni *et al*., 2008; Volpi *et al*., 2011). Similarly, transgenic durum wheat plants over-expressing constitutively TAXI-III, a member of the TAXI-type XIs, showed new abilities to control fungal xylanases in all tissues, including those that normally do not accumulate this inhibitor (Moscetti *et al*., 2013).By phytopathogenic tests we demonstrated that the over-expression of PGIP, PMEI or TAXI-IIIis effective in limiting wheat diseases caused by the fungal pathogens *Fusarium graminearum* and *Bipolaris sorokiniana*(Janni *et al*., 2008; Volpi et al., 2011; Ferrari *et al*., 2012; Moscetti *et al*., 2013). The extent of symptom reduction obtained with the over-expression of each glycosidase inhibitor varies between 25-30% for FHB caused by *F. graminearum* and about 50% for leaf blotch caused by *B. sorokiniana* (Janni *et al*., 2008; Volpi *et al*., 2011; Ferrari *et al*., 2012; Moscetti *et al*., 2013). This level of protection is similar to that observed in transgenic dicot plants expressing PGIP or PMEI (Powell *et al*. 2000; Ferrari *et al*., 2003; Agüero *et al*., 2005; Manfredini *et al*., 2006; Joubert *et al*., 2006; Ferrari *et al*., 2012; Borras-Hidalgo *et al*., 2012; Hwang *et al.*, 2010; Perez-Donoso *et al*., 2010), although the level of pectin content in wheat is much lower than in dicots (Vogel, 2008).

For wheat transgenic plants expressing PGIP, we showed also that the reduction of disease symptoms is associated with a reduced accumulation of mycotoxins and a significant reduced loss of starch accumulated in the grains compared to control plants (D'Ovidio *et al*., 2012).

Plants over-expressing constitutively TAXI-III were very useful to demonstrate, for the first time, that XIs are indeed involved in plant defence; however, its constitutive over-expression caused transgene silencing at high frequency (Moscetti *et al*., 2013), indicating that for practical application a different strategy should be considered, including the expression of XI in specific tissues or regulated by induced promoter.

Finally, the pyramiding of these three glycosidase inhibitors through co-bombardment or crossing resulted in transgene silencing at high frequencies which prevented test their combined effect on disease symptom development (Kalunke *et al*., 2013). Probably the presence of a high number of transgene copies driven by the same constitutive promoter such as *Ubiquitin1*(*Ubi-1)*, may

have triggered homology-dependent gene silencing (HDGS) (Meyer and Saedler 1996). To these unwanted results the constitutive expression of TAXI-III, normally expressed in the endosperm tissue, could have also contributed.

III – Conclusions

In conclusion, these results indicated that the host cell wall polysaccharides, irrespective of their amount and type, play a key role as functional barrier against different pathogens and that the increased accumulation of glycosidase inhibitors can contribute to maintain the integrity of the cell wall and improve wheat resistance against fungal pathogens.

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