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MILANO, ITALY
University of Milano

The brachytic 2 and 3 maize double mutant shows alterations in plant growth and embryo development

--Pilu, R; Villa, D; Cassani, E; Durante, M; Cerino Badone, F; Sirizzotti, A; Bucci A

In maize there are three types of brachytic mutants (*br1*, *br2* and *br3*) showing short stature and a gibberellins-insensitive phenotype. So far, only the brachytic 2 gene has been cloned and it encodes for a putative protein of the Multidrug Resistant (MDR) class of P-glycoproteins (PGPs) that could be involved in polar movement of auxins. With the aim of elucidating the relationship between *brachytic 2* and *3* mutations we produced by crossing the *br2 br3* double mutant and we observed for the first time a strong additive effect regarding plant stature and architecture and also an involvement of these two genes in embryo development. In fact br2 br3 plants, named “gnome”, showed shorter internodes with curled and wrinkled leaves compared to the monogenic brachytic mutation (Fig. 1). The measurement of these plants showed that gnomes are shorter by about 85% compared with the wild type plant. Starting from these gnome plants we performed four cycles of selfing to produce near-isogenic lines to use for the following studies. We noticed high sterility of these plants with the presence of a few seeds in the ears harvested. With the aim of understanding whether the *br2/br2 br3/br3* seedlings were, in some way, affected by interaction between these two brachytic mutations we germinated on paper the offspring of selfed gnome (br2 and br3 seedlings do not show any obvious difference vs wt seedlings, data not shown). We observed a strong effect regarding seedling morphology, in fact as shown in Figure 2 we identified defective seedlings roughly classified in four phenotypic classes: stunted plants like a dwarf mutant (Fig. 2B), seedlings with the first leaf that remains closed, named tube (Fig. 2C), seedlings with distorted growth (Fig. 4D) and seedlings without a shoot, named shootless (Fig. 2E). We also noticed an high level of non-germinated seeds, as reported in Table 1 where we summarize the quantitative data regarding this phenotypic distribution (taking together all the segregation data obtained from the progeny of gnome plants). We also observed a different percentage of distribution of seedling abnormalities among the different selfed *br2/br2 br3/br3* near isogenic lines developed, ranging from only non-germinated seeds to almost only stunted seedlings (data not shown). Of course the only *br2/br2 br3/br3* phenotypic class able to grow was the stunted seedlings that at maturity became gnome plants. In conclusion, the results here reported suggest for the first time, an important new role of *br2* and *br3* genes in maize embryogenesis which could contribute to answering several open questions regarding PAT and embryo development in maize and other cereals.

Table 1. Segregation of mutant seedling phenotypes obtained by selfing the *br2/br2 br3/br3* gnome plants. The seeds were germinated on imbibed paper.

cross	segregation		
br2/br2 br3/br3 selfed	gnome	abnormal seedlings	not germinated
	116 (38.66%)	49 (16.33%)	135 (45%)

Figure 1. From left to right are shown wild type, br2, br3 and gnome plants at maturity (A). (B) gnome phenotype in detail.

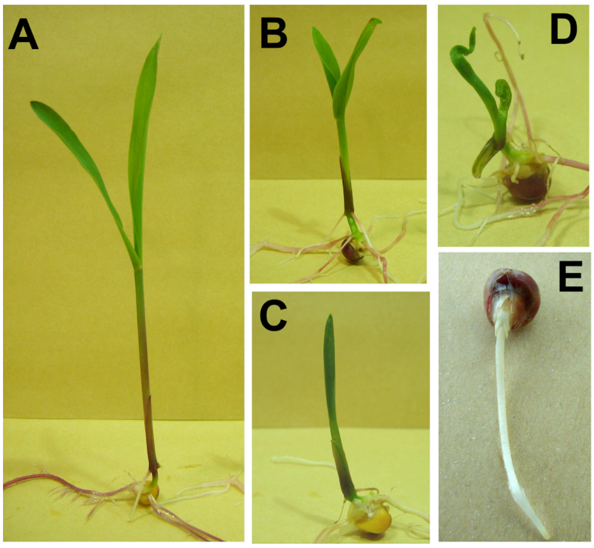

Figure 2. Range of abnormalities showed by br2br2 br3br3 seedlings. (A) wt seedling, (B) dwarfing-like seedling that will became gnome at maturity, (C) "tube" seedling: the first leaf that remains closed, (D) seedling with distorted growth, (E) shootless seedling.