A G protein alpha null mutation confers prolificacy potential in maize

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Abstract

Plasticity in plant development is controlled by environmental signals through largely unknown signalling networks. Signalling coupled by the heterotrimeric G protein complex underlies various developmental pathways in plants. The morphology of two plastic developmental pathways, root system architecture and female inflorescence formation, was quantitatively assessed in a mutant \textit{compact plant 2} (\textit{ct2}) lacking the alpha subunit of the heterotrimeric G protein complex in maize. The \textit{ct2} mutant partially compensated for a reduced shoot height by increased total leaf number, and had far more ears, even in the presence of pollination signals. The maize heterotrimeric G protein complex is important in some plastic developmental traits in maize. In particular, the maize G\textalpha subunit is required to dampen the overproduction of female inflorescences.

Key words: Cell division, development, ear, G protein, maize, signalling.

Introduction

Maize, which originated in the Tehuacan Valley of Mexico, is a large grain plant and the most widely grown crop (\textit{Long and Fritz}, 2001; \textit{Doebly}, 2004). During domestication from its ancestor (teosinte), maize decreased the number of female inflorescences, while increasing inflorescence size, grain size and number (\textit{Doebly}, 2004). Most modern maize lines, such as the B73 inbred line, initiate several ear branch shoots (shanks) per plant at successive main stalk nodes. Each shank has a single ear primordium at its tip and growth of this uppermost (‘top’ or ‘first’) ear suppresses the development of ears from lower nodes (second, third ears, etc.) on the main stalk (\textit{Pautler et al.}, 2013; \textit{Wills et al.}, 2013). Growth of the apical ear shoot also suppresses the formation of additional axillary ears on the same shank. In contrast to maize, development of productive ear shoots on branches from multiple nodes is common in teosinte, although it is occasionally observed in maize, where it is referred to as prolificacy (\textit{McClelland and Janssen}, 1929; \textit{Frank and Hallauer}, 1997; \textit{Moulia et al.}, 1999). For example, under certain conditions, maize makes multiple ear shoots on the same shank or more rarely ‘twined’ ears, defined as two separate ears with separate husks at the same node (\textit{Frank and Hallauer}, 1997). Successive nodes can also make productive (seed-bearing) ears, for example, this is common when plants are grown at lower densities. The frequency of prolificacy varies with genetic background and environmental factors, however, a molecular mechanism wiring the genetic and environmental factors remains poorly understood. In this paper, the involvement of the G protein signalling network in maize prolificacy has been reported, as well as its roles in shoot and root development.

Maize root and shoot architecture are examples of other plastic developmental traits (\textit{Brown et al.}, 2011; \textit{Yu et al.}, 2014). The juvenile maize seedling has a primary root and multiple seminal roots that originate from the subterranean...
embryo, whereas the adult plant also has crown roots emerging from aerial nodes. The overall architecture, specifically the number of each root type, lengths, and their position, is controlled by environmental cues such as the location and amount of water and nutrients in the soil profile. While maize root architecture is genetically encoded and some of these genes have been identified and studied (Hochholdinger and Tuberosa, 2009), little is known about how environmental signals are transduced to manifest root architecture (Casal et al., 2004). Like roots, shoot architecture is similarly plastic, evident by observed changes that maximize energy capture in balance with water loss. For example, planting density has a major effect on shoot architecture (Ku et al., 2015).

The heterotrimeric G protein complex, composed of α, β, and γ subunits, is an evolutionary conserved signalling complex that transmits signals from transmembrane receptors to intracellular proteins (Urano et al., 2013). A null mutation of the maize Gα gene reduces shoot growth, leading to a dwarf phenotype, ear fasciation, and thicker tassel branches (Bommert et al., 2013). Here, the nature of this phenotype has been explored in greater depth, with emphasis on the role of G protein signalling in ear development and prolificacy.

Materials and methods

Growth conditions

Seeds of wild-type B73 and the Gα-null mutant ct2 (ct2-ref) (Bommert et al., 2013) introgressed five generations into B73 were germinated and grown in 3″ soil pots for about 2 weeks in a greenhouse. The seedlings were transferred to 3-gallon pots having a diameter and height of 25 cm each. The pots were placed on a water tray of 6 cm in height filled with water two or three times a week. Nutrient contents dissolved in tap water were 250 parts per million (ppm) of nitrogen, phosphate, and potassium, 0.63 ppm of magnesium and iron, 0.31 ppm of zinc and manganese, 0.16 ppm of copper and boron, and 0.06 ppm of molybdenum. Temperature was controlled within 25.3–28.1 °C (77.5–82.5 °F) in the day and 20.5–23.3 °C (69–74 °F) at night. Experiments were conducted between April and December 2014. On days when clouds reduced the ambient irradiation below 450 W m⁻², light was supplemented with 1000 W high-intensity discharge lamps and these lamps were turned off when ambient light was above 900 W m⁻². Leaf stage (number of leaf collars), number of visible ears, height of leaf collars, and length and width of leaf blades were measured once a week. Plant height from the soil surface to the tip of the tassel was measured when tassels were fully developed.

Results and discussion

The leaf shape of the ct2 mutants was noticeably different (Fig. 1A; 5-week-old plants of B73 and ct2 grown in the greenhouse). The ct2 mutation led to a shortening of the leaf blade by 18–42% in all leaves (Fig. 1B) and plant height by 32% (see Supplementary Fig. S1A, B at JXB online), while slightly increasing leaf width (Fig. 1C). The ct2 plants also had an increased number of leaves per plant (B73, 18.4 leaves; ct2, 20.0 leaves), and a slightly delayed growth rate of leaves controlled within 25.3–28.1 °C (77.5–82.5 °F) in the day and 20.5–23.3 °C (69–74 °F) at night. Experiments were conducted between April and December 2014. On days when clouds reduced the ambient irradiation below 450 W m⁻², light was supplemented with 1000 W high-intensity discharge lamps and these lamps were turned off when ambient light was above 900 W m⁻². Leaf stage (number of leaf collars), number of visible ears, height of leaf collars, and length and width of leaf blades were measured once a week. Plant height from the soil surface to the tip of the tassel was measured when tassels were fully developed.

Root growth

B73 and ct2-ref seeds were germinated on soil for 6 d, then the seedlings were transferred to ¼× Murashige and Skoog (MS) media with 0.05% 2-(N-morpholino)ethanesulphonic acid as described previously (Urano et al., 2014). The pH was adjusted to 5.7 with potassium hydroxide. The seedlings were grown in a 24 h cycle chamber of 16 h light at 210–220 μmol m⁻² s⁻¹ and 8 h darkness at 28°C. The ¼× MS media was replaced with ½× MS media on the second week of hydroponics. The length of the longest crown root was measured on the 14th day after sowing seeds. The numbers of seminal and crown roots were counted on the 20th day.

Statistical analyses

Data were analysed by the two-tailed Student’s t test between wild-type B73 and Gα-null ct2 groups. Significant differences are shown with symbols of n.s. (not significant, P≥0.05), * (P<0.05) or ** (P<0.01).

Fig. 1. A Gα-null line decreases longitudinal growth in shoots and roots. (A) Five-week-old seedlings of B73 and the Gα-null ct2 mutant. Scale bar=20 cm. (B, C) Leaf length and width of B73 and ct2. Panels show raw values of B73 (orange dots, n=5) and ct2 (blue dots, n=4) with a curve fitted by the Gaussian distribution function. (D) Representative roots of 16-d-old B73 and ct2 seedlings grown in 2.0 l Erlenmeyer flasks. A scale shows 5 cm. (E, F, G) The longest crown root length (E) was measured on the 14th day, and number of crown roots (F) and seminal roots (G) were measured on the 20th day. Panels show raw values of B73 (orange dots, n=16) and ct2 (blue dots, n=16). Bars represent the means with standard errors of the mean. * or **, respectively, signifies a significant difference between B73 and ct2 groups at the P value less than 0.05 or 0.01, by the two-tailed Student’s t test. n.s. signifies no significant difference at the P value of 0.05. Quantitated values are presented in Supplementary Table S1 at JXB online.
(see Supplementary Fig. S1C, D at JXB online). The total leaf area of B73 was 8369 cm² (n=5), compared with ct2 total leaf area of 6515 cm² (n=4), 22% less than the wild type. Thus the increased number of leaves in ct2 mutants did not fully compensate the reduced individual leaf area.

The ct2 mutation also reduced root growth and crown root formation (Fig. 1D–G). Figure 1D shows B73 and ct2 roots grown hydroponically for 2 weeks. The ct2 mutant had fewer seminal roots, and fewer and shorter crown roots, as quantified in Fig. 1E–G. These results suggested that a Gα signalling network modulates cell proliferation both in shoots and roots, although the effect by Gα-null mutation was greater on the shoot than the root system (shoot, 32% reduction; root 11% reduction).

In addition to the dwarf defect, we observed ct2 plants having multiple ear shoots on a single shank (Fig. 2; see Supplementary Fig. S2 at JXB online). The axillary ear shoots were smaller and had poor kernel fill. Supplementary Fig. S2A, B at JXB online show representative stalks of B73 and ct2 at the 14th week. Both B73 and ct2 plants usually exhibited one or two visible ear shanks, each with a single ear at the apex, when the uppermost ear was pollinated. However, about 15% of ct2 plants, while none of the B73 plants, formed several axillary ear shoots on the uppermost shank (Fig. 2B). Because poor kernel fill is associated with the multiple ear formation trait (McClelland and Janssen, 1929), pollination was inhibited and axillary ear formation was analysed (Fig. 2A; see Supplementary Fig. S2 at JXB online). While most B73 plants still exhibited a single ear on a shank under the non-pollinated condition, the uppermost ear node of ct2 formed multiple visible axillary ears, as indicated by arrowheads (Fig. 2A). Figure 2B and Supplementary Fig. S2C, D at JXB online provide the quantitation of this phenotype. Therefore, ct2 mutants, when unpollinated, had more visibly-developed ears per plant (B73, mean 4.1 ears; ct2, 7.8 ears), and on the uppermost node of the main stalk (B73, mean 1.3 ears; ct2, 3.9 ears). Inhibition of pollination similarly promoted development of ear shanks at lower nodes on the main stalk (Fig. 2C), however, no difference was observed between the B73 and ct2 groups (B73, mean 3.8 nodes forming a visible ear shank; ct2, 3.9 nodes). Prolificacy was not observed in pollinated groups of B73 or ct2 (Fig. 2B, C), suggesting that it requires both low pollination and mutation of ct2.

Low pollination of ct2 caused axillary ear formation two or more weeks after the apical ear emerged (see Supplementary Fig. S2C, E at JXB online), probably by releasing them from growth arrest. Because the ct2 mutation showed an additive effect with low pollination, it was predicted that more female inflorescences were formed on ct2 mutant shanks. Therefore, ear shoots were dissected and all mature and immature female inflorescences of B73 and ct2 were counted (Fig. 3), and it was found that B73 had few axillary inflorescences (B73 with pollination, mean 0.43 axillary ears; B73 without pollination, 0.57 axillary ears) (Fig. 3F; see Supplementary Table S2 at JXB online). These axillary ear shoots aborted when the apical ear shoot was pollinated (Fig. 3A), but elongated when the apical ear had not been pollinated (Fig. 3B). The ct2 mutant increased the number of axillary ear shoots (Fig. 3D–F) and occasionally exhibited secondary axillary ear shoots from the axillary ears (indicated by red arrowheads in Fig. 3E and in Supplementary Fig. S3 at JXB online). Pollination did not affect the number of inflorescences on the uppermost ear shank (ct2 with pollination, mean 5.0 ears; ct2 without pollination, 5.7 ears), but low pollination allowed them to elongate, as observed for B73 (Fig. 3B, E). These results indicate that the ct2 mutation allowed more prolific formation of axillary ear shoots, while low pollination caused a general release of the axillary ear shoots from growth arrest.

Figure 4 shows a two-step model for conferring prolificity. Genetics studies identified additional genes affecting the ear formation trait. Activation of a transcription factor, BARREN STALK1 (BA1), initiates the axillary ear shoot meristems, while another transcription factor gene, GRASSY TILLERS1 (GT1), suppresses the outgrowth of immature inflorescences (Ritter et al., 2002; Gallavotti et al., 2004; Whipple et al., 2011). A different expression profile of GT1 in the nodal plexus probably caused a distinct ear branching pattern between maize and teosinte (Wills et al., 2013). Our results prompt the speculation that the G protein network regulates axillary meristem initiation/transition of axillary buds to reproductive development and/or outgrowth of immature ear shoots (Fig. 4), so may control these transcription factors. Although the signalling mechanism regulating these and other genetic components remains poorly understood.

Fig. 2. The ct2 Gα-null mutant forms multiple ears at a single node. (A) The main stalk of unpollinated wild-type B73 and Gα-null ct2 mutant. Red arrowheads point to apical ears with silks. Yellow arrowheads indicate axillary ears formed on the uppermost (top) ear shank. (B, C) Number of ears formed on the uppermost ear shank or on all nodes having ears. Data were collected from 15-week-old B73 and ct2 plants. Graphs in (B) and (C) present raw values of B73 (blue dots) and ct2 (orange dots), the means, and the standard errors. ** Represents significant difference between B73 and ct2 groups at the P value less than 0.01 by the Student’s t-test. n.s. signifies no significant difference at the P value of 0.05. n.a. Represents not statistically analysed, because all the values of the B73 or ct2 group were identical. Quantitated values are available at Supplementary Table S2 at JXB online. See Supplementary Fig. S2 at JXB online for other images for wild-type B73 and Gα-null ct2 plants.
understood, it is empirically known that ear outgrowth requires ample energy resources—water, light, and nutrients (Lejeune and Bernier, 1996; Moula et al., 1999; Markham and Stoltenberg, 2010). Multiple hormones—auxin, cytokinin, and strigolactones—also regulate the dormancy of axillary buds (Pautler et al., 2013). Maize and other plant G-protein networks couple those extracellular stimuli and modulate meristem activity, cell proliferation, and cellular senescence (Bommert et al., 2013; Urano et al., 2013; Sun et al., 2014), therefore G protein signalling may bridge these extracellular signals to ear development and outgrowth. The phenotypes described here, including root system and ear shoot architecture, are obvious plastic traits in plant development that have been selected during crop domestication and our results suggest that G protein signalling networks modulate the expression of these key agronomic traits. It will also be interesting to ask how natural variation in G protein signalling components has contributed to crop improvement.

**Supplementary data**

Supplementary data can be found at *JXB* online.

**Supplementary Fig. S1.** Vegetative growth of B73 and Gα-null ct2 lines.

**Supplementary Fig. S2.** Ear formation of B73 and Gα-null ct2 lines.

**Supplementary Fig. S3.** Apical and axillary ears of a representative ct2 plant.

**Supplementary Table S1.** Shoot and root growth of B73 and Gα-null ct2 lines.

**Supplementary Table S2.** Female inflorescence formation in B73 and Gα-null ct2 lines.
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