Short Communication

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A GRAFTING-INDUCED SORGHUM-MAIZE HYBRID

SUMMARY

Interspecific grafting is limited by graft-incompatibility but regeneration of hybrid plants from grafting of genetically distinct scions and rootstocks has been reported in tobaccos. Here we report the regeneration of a hybrid plant via grafting between cross-incompatible sorghum and maize. By grafting maize and sorghum plants and regenerating hybrid from the graft junction in selection media, we generated a plant that was phenotypically a hybrid between sorghum and maize, i.e., the hybrid produced a seed-bearing "ear" toward the top of the stalk. To our knowledge, this is the first report of successful hybrid from grafting between two monocot plants.

Keywords: sorghum, maize, grafting, regeneration, hybrid

INTRODUCTION

Grafting is a common horticulture technique that has been in practice for over 2000 years. Use of grafting has been referenced in Bible, ancient Greek and Chinese text, indicating its practice by at least 5th century BCE (Melnyk and Meyerowitz 2015). Grafting has been important in agriculture and horticulture for centuries and some of the major benefits from grafting include domestication of woody fruit plants such as apples, pears, and plums (Mudge *et al*. 2009), asexual propagation of desirable plants as well as introduction of resistance to various biotic and abiotic stresses (Lee *et al*. 2010) and changes in growth habits of scion by altering its characteristic such as size, growth vigor, and fruit yield (Lee *et al.* 2010, Mudge *et al*. 2009).

Similar to sexual hybridization, there is also graft incompatibility between species. In sexual hybridization, due to reproductive barriers interspecific hybrids between cross-incompatible parents are not viable because of gametic

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incompatibility (prezygotic) and hybrid breakdown (postzygotic incompatibility) (Bates and Deyoe 1973). Similarly, there is also grafting-incompatibility, i.e., majority of plants will graft to themselves, fewer will graft to very closely related species, and only rarely will plants successfully graft to more distant relatives (Melnyk and Meyerowitz 2015). In eudicot compatible grafting at graft junction, ruptured cells collapse and the intact cells adhere to the opposing tissue right after grafting. Subsequently, cell divides to give rise to phloem and xylem, followed by the formation of plasmodesmata and cytosolic channels between cells across the junction. In eudicot incompatible grafting, cell still divides at the junction but phloem and xylem differentiation may not occur. In both cases, aligning vascular cambia between scions and rootstocks improves grafting success rate (Melnyk and Meyerowitz 2015).

Although grafting in eudicots is common and has been in use for centuries, grafting between monocots is more difficult as monocots have scattered vascular bundles and do not have a vascular cambium, but can be grafted by aligning meristem tissues from scion and rootstock although success rate may still be low (Muzik and La Rue 1952, 1954). This explains little research reported in the literature dealing with monocot grafting, if any they focus on greenhouse setting grafting. Muzik and La Rue (1952, 1954) had a 3% graft success rate while grafting monocot plants. Successful grafts generated between para grass (*Panicum purpurascens* Raddi.) and merker grass (*Pennisetum purpureum Schum*. var. *merkeri*) grew and set seeds. However, grafting of monocots in tissue culture setting has not been reported. Monocots such as maize, rice, and sorghum are some of the economically most important crops. Grafting of such commercially important plants to improve their agronomic traits might in long term be economically more beneficial. Successful grafting although less frequent could provide monocot plants with genetic variation that will help improve traits such as growth rate, size, yield, environmental stress tolerances and more.

Grafting involves the physical connection between the shoot of one plant (scion) and the rooted part of another (rootstock) plant. After grafting, plants respond rapidly by activating the wound healing and begin the regeneration process (Melknyk, 2017). The wound healing process might be activated by the disconnection between leaves and the roots by changing the transport dynamics (Friml and Palme 2002) or by detection of damages to cell in the graft region and subsequent triggering of the plant defense and growth responses (Nushe, 2012). Graft healing leads to regeneration of tissues around the wound. Normally, grafting creates a compound genetic system by uniting two or more distinct genotypes, each of which maintains its own genetic identity throughout the life of the grafted plant (Mudge *et al*. 2009), but the closeness of cells from the two genotypes allows movement of nuclei through plasmadamata in a cytomixis-like process (Fuentes *et al.* 2014). This produces hybrid cells with chromosomes from both scion and rootstocks and hybrid plants after regeneration. This process can serve as a route to generate asexual allopolyploid hybrids (Fuentes *et al*. 2014, Stegemann and Brock 2009, Stegemann *et al*. 2012).

Grafting to produce new species of plant is relatively new. Although allopolyploidization in plants is common and it leads to success of crop domestication as well as speciation and environmental adaptation, cross species allopolyploidization through asexual mechanism is very rare. In tobacco, the grafting between herbaceous (*Nicotiana tabacum*) and woody (*Nicotiana glauca*) tobaccos produced hybrid plant from graft junction which had the genomes from both rootstock and scion and was named *Nicotiana tabauca*. The allopolypoid hybrid was a result of migration of nuclei from cell to cell at graft junction through plasmodesmata in a cytomixis-like process, not through cell fusion (Fuentes *et al.* 2014). The movement of the entire nuclear genome across the graft junction thus raises the possibility of generating new plant species which has the characters of both parents and might lead to the generation of economically important hybrid plants. In this study, we demonstrated that graft-induced hybrids can be produced between monocot scions and rootstocks.

MATERIAL AND METHODS

Plant materials: Surface sterilized transgenic maize (resistant to phosphinothricin) and sorghum (resistant to hygromycin) seeds were grown under aseptic conditions by germinating in Magenta® box with MS Medium supplemented with 3% sucrose. Plants were grown under diurnal cycle of 16 hours light and 8 hours of dark at 25°C.

Grafting: grafting experiments were performed using sterile transgenic plants under aseptic condition in the laminar air flow hood. Stems of similar sized transgenic maize and sorghum plants were cut at approximately 45-degree angle. The scion and rootstock were joined and held together using sterile silicon tubes. The reciprocal grafting was done with each plant serving as both rootstock and scion, giving rise to two grafts. These grafts were grown in MS media supplemented with 3% sucrose and 1 mg/L 2,4-D (2, 4- dichlorophenoxyacetic acid) for 2 weeks at 25°C with 16 hrs light and 8 hrs dark photo period.

Selection and regeneration of hybrid: After 2 weeks, graft site was excised and exposed to regeneration medium containing 50 mg/l hygromycin and 3 mg/l phosphinothricin. The regeneration medium was also supplemented with 3.5 mg/l BAP, 0.2 mg/l IBA and 0.2 mg/L kinetin. Successful selection was defined as the growth of callus followed by production of shoots from the graft region. Plants so produced were transferred to regeneration medium with double selection (phosphinothricin and hygromycin) to produce longer shoots and roots.

Putative hybrid plant was transferred to soil after regeneration of roots in regeneration media followed by 3 days of gentle acclimatization to open air. The plants in soil were transferred to greenhouse and grown at 30°C with natural daylight with periodic watering and fertilization.

RESULTS AND DISCUSSION

Over 850 grafts were made between transgenic sorghum and transgenic maize plants in aseptic conditions. Reciprocal cleft grafting was used in the

majority of grafts; however, in some cases where maize stem size was bigger than sorghum, maize was used as the rootstock and sorghum as scion (Figure 1).

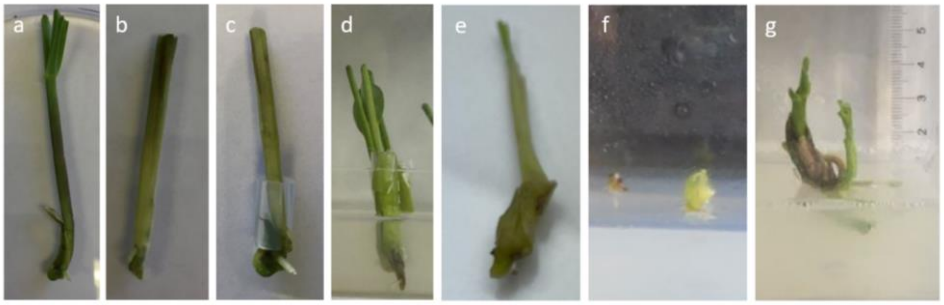

Figure 1. Different stages of monocot grafting in vitro: (a) Maize (b) Sorghum (c) Graft with tube (d) Graft in Ms media day 1 (e) Graft after 15 days tube removed (f) graft in regeneration for 3 weeks (g) Graft in regeneration for 5 weeks

Silicon tubes pre-sterilized in bleach were used to hold the grafted plants in place and they worked better than paper clips or copper wire and were easy to use. After 2 weeks of incubation in MS media supplemented with 2,4-D, graft junctions were cut in sterile environments and transferred to selection media. A total of 358 graft junctions (42% of 850) that were properly connected and still living were transferred to selection media. In the following two weeks the graft junctions were under double selection of hygromycin and phosphinothricin in which 205 of them died. The 153 surviving graft junctions were transferred to regeneration media with double selection. Regeneration media was changed every 3 weeks to supply fresh nutrients and selection. Following multiple subcultures on regeneration media the number of surviving graft junction with calli was reduced to 30. From these only 6 plantlets (0.59%) regenerated and grew---we also used germinating sorghum and maize seeds for grafting as in Reeves et al. (2022) but failed to generate any hybrids (data not shown). These six plantlets were transferred to rooting media with double selection, on which 2 more died. Out of remaining four, two were able to produce the roots and thus transferred to soil following acclimatization for 3 days and grown in greenhouse. One plant died after 2 weeks whereas other one started to grow. Other two plantlets in rooting media were subcultured multiple times but died eventually.

The putative hybrid plant looked like sorghum at first: smooth green leaves with a thin stem. But growth stalled after 3 weeks and after another week a new stem started to grow. This time the plant started to show leaf features of maize plant while the original stem died off. After a month the plant was around 10 cm tall. We noticed a seed-bearing ear growing in the stalk toward the top of the plant. This indicated the plant although very small was mature enough to produce a “corn ear” as part of the stalk, not as “ears” on the stalk (Figure 2).

Figure 2. Hybrid plant at different growth stages: (a) Hybrid in soil at week 1. (b) Hybrid in soil at week 4. (c) Hybrid at week 7.

Mobility of DNA between scions and rootstocks in graft junction has been demonstrated in recent years. For example, it has been demonstrated that plant grafting can result in the exchange of genetic information via either large DNA pieces or entire plastid genomes (Stegemann and Bock 2009), complete chloroplast genomes can travel across the graft junction from one species into another (Stegemann *et al*. 2012) and complete nuclear genomes can travel across the graft junction from one species into another (Fuentes *et al.* 2014). From these studies, the group were able to generate graft-induced hybrids (Fuentes *et al.* 2014). Fuentes *et al.* (2014) were the first to report hybrid plant (*Nicotiana tabauca*) that combined nuclear genomes of *N. glauca* and *N. tabacum*. These results indicate the transfer of nuclear genomes across the graft junction that produces hybrids.

In vitro grafting successes of under 1% in our study although lower than expected is significant in the sense that we managed to grow it from the graft junction between two monocot plants. This implies the transfer of genomic DNA between two plants that produced a new hybrid. This hybrid plant being resistant to both hygromycin and phosphinothricin demonstrates the presence of DNA from both maize and sorghum genomes, although the plant was much smaller than its scion/rootstock parents (Figure 2) due to hybrid weakness (Chen *et al*. 2014). The hybrid plant had a seed-bearing ear close to the position of maize tassel or sorghum panicle. This may be due to subgenome dominance of maize in the hybrid as demonstrated in allotetraploid *Senecio mohavensis* (Alexander-Webber *et al*. 2016) in which the allopolyploid hybrids preferentially express genes from one parent with corresponding phenotypic consequences. In our hybrid, the maize genome may have a gene expression bias in its favor although this is to be confirmed. By producing the hybrid from the graft junction in vitro we demonstrated in this study that different species of monocot plants not only can be grafted but also their nuclear genomes be transferred through graft

junction to produce a hybrid. It also showed that the sexually incompatible sorghum and maize (Bernard and Jewell 1985) are graft-compatible although with the characteristic low success rate of monocots.

CONCLUSIONS

In this study, we grafted phosphinothricin-resistant maize and hygromycin-resistant sorghum plants in vitro and regenerating hybrid from the graft junction in selection media. We generated a plant that was phenotypically a hybrid between sorghum and maize, i.e., the hybrid produced a seed-bearing "ear" toward the top of the stalk. To our knowledge, this is the first report of successful hybrid from grafting between two grass species.

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