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New insight in the Gibberellin biosynthesis and signal transduction

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Gibberellin (GA) plays important roles through plant growth and development. However, where GA is synthesized inside a cell and how it regulates sex determination is obscure. We analyzed the classic *dwarf1* (*d1*) mutant in maize and revealed that D1 encodes GA 3-oxidase converting inactive GA intermediates to bioactive GA. As such, the D1 protein marks the sites where GA is potentially synthesized. Interestingly, the D1 protein was found to localize in the cytosol and nucleus, a dual-localization coinciding with the GA receptor. The same result was found for GA 20-oxidase catalyzing the upstream reaction. These results suggest that GA can be synthesized in the cytosol and nucleus. The D1 protein was highly and specifically expressed in the stamen primordia in the ear florets, but low in the whole tassel. Hence it is possible that low level of GA in the tassel is insufficient to suppress stamen development. As jasmonic acid (JA) plays antagonistic role to GA in the tassel florets, here we propose a model to explain this antagonism effect on the regulation of the stamen and pistil organ development in the tassel florets in maize.

Keywords: gibberellin, GA 3-oxidase, maize, nucleus and cytosol, sex determination

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Addendum to: **The Maize *DWARF1* Encodes a Gibberellin 3-Oxidase and Is Dual-Localized to the Nucleus and Cytosol**
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Introduction

Gibberellin (GA) plays many important roles in plant growth and development such as promoting seed germination and stem elongation, regulating flower development. GA biosynthesis is believed to occur in 3 compartments within the cell.[1] The first phase from geranylgeranyl diphosphate (GGDP) to *ent*-kaurene occurs in plastids; the second phase from *ent*-kaurene to GA_{12} in the plastid envelope and endoplasmic reticulum (ER); and the final phase from GA_{12} to bioactive GAs in the cytosol (**Fig. 1**).[1] In contrary to this widely accepted conception, our recent study indicated that bioactive GA formation may be carried out in 2 compartments, the cytosol and the nucleus. Interestingly, this dual-localization coincides with the localization of GA receptor GID1.[2,3]

Maize carries unisexual flowers, male flowers (tassel) on top of the plant and female flowers (ear) in the axial of the leaf. In fact, maize develops bisexual flowers in both the tassel and the ear at early developmental stages.[4] The unisexuality is achieved by selective suppression of pistil primordia in the tassel and the stamen primordia in the ear.[4] The suppression of stamen primordia in the ear is attributed to GA, whereas the sexuality in the tassel florets is attributed to the antagonism between GA and jasmonic acid (JA). As the unisexual system is derived from bisexual system, unraveling the molecular mechanism of sex determination is important for understanding the evolution of flowers and plants.

The maize *DWARF1* encodes a GA 3-oxidase

The maize *dwarf1* (*d1*) mutant is a classic mutant known for decades.[5,6] Genetic and biochemical analyses indicate that GA 3-oxidase (GA3ox) activity is absent in the *d1* mutant,[7] but the molecular basis for this mutation has not been revealed. Based on existing evidence, 2 possibilities were put forward: 1) D1 encoded a GA3ox that was directly involved in the GA biosynthesis; 2) D1 encoded a regulatory protein that was required for GA3ox expression or enzymatic function. Previous study mapped

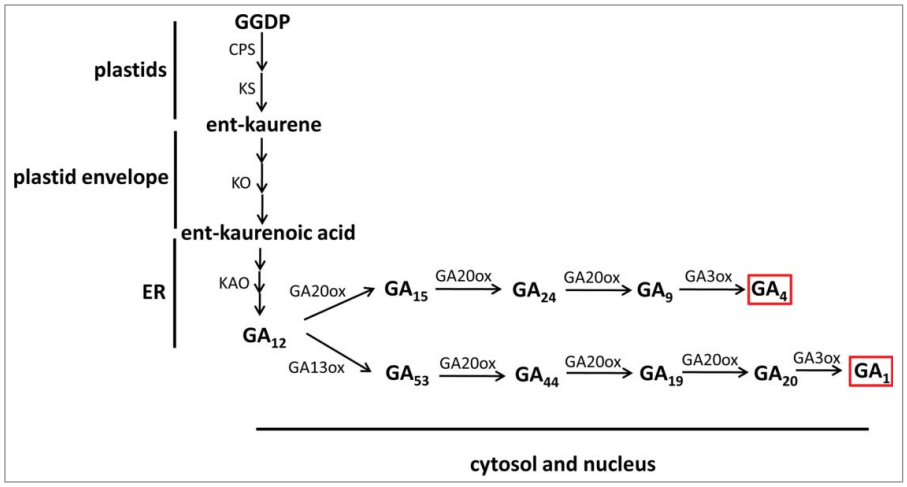

Figure 1. The GA biosynthetic pathway. Red rectangle indicates bioactive forms.

d1 on chromosome 3S;[8] which was in the proximity of a putative GA3ox gene, hence strengthening the first possibility. By analyzing 4 *d1* alleles, we revealed that each of the alleles contained mutation in ZmGA3ox2 gene. The mutation in *d1–6016* allele was also independently found in GA3ox2 recently.[9] Thus, these results confirmed that ZmGA3ox2 is the causative gene for *d1* phenotype. Enzymatic analysis of D1 protein indicated that it can catalyze at least 4 reactions leading to bioactive GA formation: GA_9 to GA_4, GA_{20} to GA_1, GA_{20} to GA_3, and GA_5 to GA_3. These results confirm that *D1* encodes a GA 3-oxidase converting GA intermediates to bioactive GAs. The maize genome contains another putative GA 3-oxidase ZmGA3ox1. However, expression of this gene was not detected, indicating that ZmGA3ox2 (D1) provides the predominant GA 3-oxidase activity in maize. This conclusion is also supported by the severe GA-deficient phenotype of *d1*.

GA can be synthesized in the nucleus and cytosol

The functions of GA20ox and GA3ox are required for the conversion from GA_{12} to bioactive GAs.[1] Because of the lacking of apparent targeting sequences, these 2 proteins were believed to be localized in cytosol.[1] However, by using 2 independent approaches, i.e. the GFP fusion and Western blot analysis on isolated organelles, we uncovered that both D1 and ZmGA20ox1 are dual-localized in the cytosol and the nucleus. This dual-localization is consistent with the dual-localization of GA receptor GID1.[2,3] Because the D1 localization determines the potential sites of bioactive GA production, these results suggest that bioactive GA may be synthesized in both the cytosol and nucleus, although further experiments are required to fully confirm this conclusion. Nonetheless, our finding provides new insight to the understanding of the GA biosynthesis and perception in plants. However, several important questions invite attention:

One) Why do these enzymes co-exist in 2 compartments?

Two) How do cells regulate the expression of the enzymes in 2 compartments?

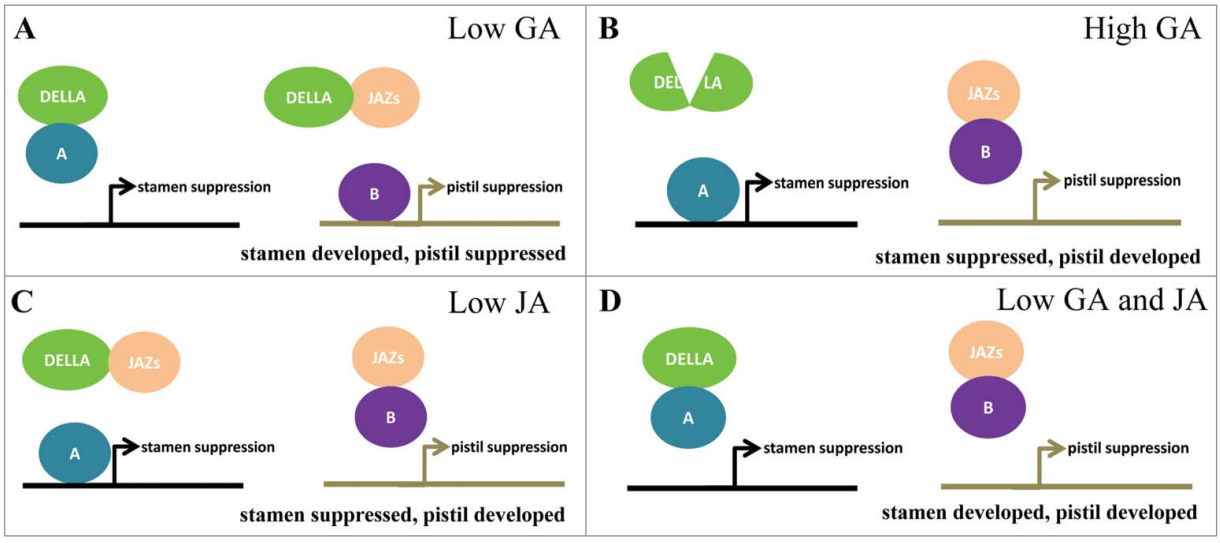

Figure 2. The model of GA and JA crosstalk through direct interaction in regulating tassel floret development. (**A**) When GA level is low (e.g. WT and *d1* tassel florets), abundant DELLA proteins restrict protein A to suppress stamen development and interact with JAZs to release some protein Bs to suppress pistil development. (**B**) When GA level is high (e.g., exogenous GA in WT tassel florets), DELLA proteins are degraded and protein As are released to suppress stamen development; while JAZs restrict protein B to suppress pistil development. (**C**) When JA level is low (e.g. *ts1* or *ts2* mutant), abundant JAZs restrict protein B to suppress pistil development and interact with DELLA to release some protein As from DELLA to suppress stamen development. (**D**) When both GA and JA levels are low (e.g., *ts2 d1* double mutant), DELLA and JAZs restrict protein A and protein B, respectively. The development of stamen and pistil are not suppressed.

Three) Will GA be transported between cytosol and nucleus?

Four) Are there new components involved in the GA perception in these 2 compartments?

GA is antagonistic to JA in regulating stamen development in the tassel

We found that the D1 protein was highly expressed in the stamen cells of ear florets, but undetectable in the tassel with the same antibody. Although the mechanism is unknown, this result together with the genetic evidence invites the notion that high level of GA in specific cells suppresses cell growth and differentiation; whereas low level of GA promotes cell growth and differentiation. This notion is supported by the evidences: 1) the level of GA in the tassel is much lower than in the ear;[10] 2) application of GA converts male florets to female florets in the tassel.[11] Thus, we postulate that high level of bioactive GA suppresses the stamen development in tassel florets. Interestingly, JA acts antagonistically to GA in regulating stamen cell development.[4] This antagonism may be indirect or direct though affecting the synthesis and/or the signaling.

In *Arabidopsis*, GA and JA crosstalk through direct interaction between the core signaling repressors DELLA (GA) and JAZs (JA).[12] Inspired by this finding, we proposed a model to explain the crosstalk of GA and JA in regulating stamen and pistil development in tassel florets. Protein A is a DELLA interacting protein and suppresses stamen development; protein B is a JAZs interacting protein and suppresses pistil development (**Fig. 2**). GA induces DELLA degradation to release protein A's function; while JA induces the degradation of JAZs to release protein B's function. When GA level is low, abundant DELLA will competitively bind to JAZs so that some protein Bs are released from JAZs to suppress pistil development. When JA is lacking, abundant JAZs will competitively bind to DELLA resulting in some free protein As to suppress stamen development. The identification of protein A and protein B will be a breakthrough for unraveling the regulation mechanism of maize sex determination in the future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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