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Gibberellin and rice heterosis

To whom all correspondence should be addressed:

Qifa Zhang, Ph.D.

Professor and Director

National Key Laboratory of Crop Genetic Improvement and National Centre of Plant Gene Research (Wuhan)

Huazhong Agricultural University

Wuhan 430070, China

Tel: +86-27-87282429, Fax: +86-27-87287092

E-mail: qifazh@mail.hzau.edu.cn

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Title

Heterosis in rice seedlings: its relationship to gibberellin content and expression of gibberellin metabolism and signaling genes

Authors

Qian Ma, Peter Hedden, Qifa Zhang*

Affiliations

National Key Laboratory of Crop Genetic Improvement and National Centre of Plant Gene Research (Wuhan), Huazhong Agricultural University, Wuhan 430070, China (Q.M., Q.Z.)

Centre for Crop Genetic Improvement, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom (P.H.)

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*Author for correspondence; email qifazh@mail.hzau.edu.cn

ABSTRACT

Despite the accumulation of data on the genetic and molecular understanding of heterosis, there is little information on the regulation of heterosis at the physiological level. In this study, we performed a quantitative analysis of endogenous gibberellin (GA) content and expression profiling of the GA metabolism and signaling genes to investigate the possible relationship between GA signaling and heterosis for seedling development in rice. The materials used were an incomplete diallel set of 3×3 crosses and the six parents. In the growing shoots of the seedlings at 20 d after sowing, significant positive correlations between the contents of some GA species and performance and heterosis based on shoot dry mass were detected. Expression analyses of GA-related genes by real-time RT-PCR revealed that 13 out of the 16 GA-related genes examined exhibited significant differential expression among F_1 hybrid and its parents, acting predominantly in the modes of over-dominance and positive dominance. Expression levels of nine genes in the hybrids displayed significant positive correlations with the heterosis of shoot dry mass. These results imply that GAs play a positive role in the regulation of heterosis for rice seedling development. In shoots plus root axes of 4-d-old germinating seeds that had undergone the de-etiolation, mimicking normal germination in soil, the axes dry mass was positively correlated with the content of GA_{29}, but negatively correlated with that of GA_{19}. Our findings provide supporting evidence for GAs playing an important regulatory role in heterosis for rice seedling development.

INTRODUCTION

Heterosis or hybrid vigor refers to the phenomenon in which hybrids outperform their inbred parents in yield, biomass, biotic and abiotic stress tolerance, or other traits. Heterosis has been widely exploited to increase productivity of crop plants for many decades (Stuber, 1994; Yuan, 1998). Generally, the life cycle of rice plants can be divided into vegetative and reproductive stages. In previous studies, heterosis was demonstrated in yield and yield component traits of rice (Zhang et al., 1994; Xiao et al., 1995; Yu et al., 1997; Li et al., 2001; Luo et al., 2001; Hua et al., 2003), but very few studies have been conducted to systematically examine heterosis in seedling traits, which is the basis for canopy development and yield.

The biological basis of heterosis has been of primary interest for biology researchers for many years due to its scientific and practical significance. Complete elucidation of the mechanism for this phenomenon requires knowledge at three levels: genetic, molecular and physiological. Genetic models of heterosis currently rely on information from QTL analyses of yield-related traits in various plant species, which show that dominance, overdominance and epistasis are all involved in the manifestation of heterosis, with various degrees of significance according to experimental design and different species (Stuber et al., 1992; Xiao et al., 1995; Yu et al., 1997; Hua et al., 2002, 2003; Kusterer et al., 2007; Melchinger et al., 2007, Li et al., 2008; Garcia et al., 2008).

To unravel the molecular basis of heterosis, high throughput expression profiling of heterotic crosses in various plant species was carried out, which identified large numbers of differentially regulated genes with diverse functions (Guo et al., 2006; Huang et al., 2006; Swanson-Wagner et al., 2006; Zhang et al., 2008; Wei et al., 2009; He et al., 2010). Although no consensus results have emerged, it is thought that allelic variants at a large number of loci acting through partial to complete dominance would provide favorable combinations resulting in superior hybrid phenotypes (Springer and Stupar, 2007). In addition, allelic variations in genic sequences, genome structure, DNA methylation patterns and chromatin structure also contribute to the molecular basis of heterosis (Springer and Stupar, 2007).

In contrast, knowledge regarding the physiological basis of heterosis is sporadic and is mainly focused on some specific traits, such as freezing tolerance in *Arabidopsis* (Korn et al., 2010). With the development of the technologies for high throughput metabolic profiling, it is now possible to predict heterosis using both DNA and physiologically metabolic markers (Gärtner et al., 2009; Andorf et al., 2010), which will be of considerable value for finding physiological clues for heterosis.

Gibberellins (GAs) are a group of tetracyclic diterpene phytohormones that control diverse aspects of plant growth and development, from seed germination, leaf expansion, and stem elongation to flower initiation and the development of flowers and fruits (Davies, 2004; Fleet and Sun, 2005). Bioactive GAs are synthesized from the common diterpene precursor geranylgeranyl diphosphate (Fig. 1), which is first converted to *ent*-kaurene by two kinds of diterpene cyclases, *ent*-copalyl diphosphate synthase (CPS) and *ent*-kaurene synthase (KS), followed by sequential oxidations to produce GA_{12}, catalyzed by two cytochrome P450 monooxygenases, *ent*-kaurene oxidase (KO) and *ent*-kaurenoic acid oxidase (KAO). At the final stage of bioactive GA synthesis, GA_{53} / GA_{12} is converted to GA_1 / GA_4 by two 2-oxoglutarate-dependent dioxygenases (2ODDs), GA 20-oxidase (GA20ox) and GA 3-oxidase (GA3ox). Deactivation of bioactive GAs and their precursors is catalyzed by another dioxygenase, GA 2-oxidase (GA2ox), and a P450 monooxygenase, EUI (Yamaguchi, 2008). GA signaling proceeds by de-repression of DELLA protein, a repressor of GA signaling, with the aid of a GA receptor GID1 and an F-box protein GID2 (Sasaki et al., 2003; Ueguchi-Tanaka et al, 2005). In addition, the α-subunit of the heterotrimeric G protein (Gα) is thought to be a positive regulator of GA signaling (Ueguchi-Tanaka et al., 2000). In recent years, the aforementioned GA metabolism and signaling genes have been cloned in rice (Sakamoto et al., 2004; Zhu et al., 2006; Lo et al., 2008), and these genes are regulated by development, hormones, light, temperature and stress (Yamaguchi, 2008).

There have been studies in the role of GAs in the regulation of heterosis. For example, Rood et al. (1988) analyzed the differences in responsiveness to the exogenous application of GA_3 and endogenous levels of GAs between F_1 hybrids and

their inbred parents of diallel combinations in maize. They found that inbreds were more responsive than the hybrids to the exogenous GA_3, while the hybrids had higher concentrations of endogenous GAs than their parental inbreds. They concluded that the increased endogenous concentration of GA in the hybrids could provide a phytohormone basis for heterosis for shoot growth.

Plants maintain the levels of bioactive GAs via feedback and feedforward regulation of GA metabolism (Hedden and Phillips, 2000; Olszewski et al., 2002), and the dioxygenases, namely GA20oxs, GA3oxs and GA2oxs, are the main targets of regulation by GA signaling to establish homeostasis. For example, an elevated level of bioactive GA usually suppresses the expression of *GA20ox* and *GA3ox*, while stimulating the expression of *GA2ox*; conversely, a drop in bioactive GA level usually up-regulates the expression of *GA20ox* and *GA3ox*, down-regulates the expression of *GA2ox*. This kind of delicate homeostatic balance of GA metabolism makes it difficult to accurately interpret results of individual experiments with respect to the physiological implications of changes in GA-biosynthetic gene expression, which reinforces the necessity of measuring GA contents not only for the bioactive hormones, but also for their precursors and catabolites.

In this study, we investigated the possible role of GAs in the regulation of heterosis in rice. Using an incomplete diallel set consisting of six elite inbreds and corresponding hybrids with varying heterotic response, we measured the amounts of both bioactive GAs and their precursors and catabolites, and analyzed the expression levels of GA metabolism and signaling genes. The results showed positive correlations between not only several GA species but also some GA-related genes and rice seedling heterosis.

RESULTS

Heterosis of Rice Seedlings and Germinating Seeds

A survey of the occurrence and the degree of heterosis related to rice seedling growth was conducted with the incomplete diallel set of 30 F_1 hybrids and their 11 parents, including five maintainers and six restorers. A number of seedling growth traits were

assayed at 20 and 30 d after sowing (DAS, five to ten individuals per genotype) for the 41 entries (Supplemental Table S2). Shoot dry weight and tiller number per seedling exhibited high level of heterosis, especially at 30 DAS. A derived trait, relative growth rate, based on shoot dry weight at two stages, also showed high heterosis. The distribution of mid-parent heterosis of the three traits for rice seedling growth is shown in Fig. 2A-C. At 20 DAS, very little or even moderate negative heterosis was observed for shoot dry weight and tiller number in some of the crosses. In contrast, high level of heterosis was observed at 30 DAS for shoot dry weight in the majority of the 30 crosses and for tiller number in all of the crosses. Mid-parent heterosis (MPH) ranged from -9% to 61.7% for shoot dry weight and 1.4% to 73.3% for tiller number. Two crosses, Maxie × Minghui 63 and II-32 × 6078, expressed ≥ 50% MPH for both traits. There was a highly significant correlation between shoot dry weight and tiller number in both F_1 performance (r = 0.577, P < 0.01) and heterosis (r = 0.724, P < 0.01), indicating contribution of heterosis to the F_1 performance at this stage.

From the original incomplete diallel set, three restorer lines (Ce 64, Minghui 63, Mianhui 725) and three maintainer lines (Jin 23, Maxie, Zhenshan 97) were selected and intermated with each other (3 × 3) to form a subset of incomplete diallel of nine crosses to represent low, intermediate and high levels of seedling heterosis for GA quantification and gene expression analyses. The measurements of shoot dry weight, tiller number and heterosis of these genotypes are presented in Tables 1 and 2.

Since seed germination is also an important trait, we also analyzed seed germination in F_1 hybrids and parents of the 3 × 3 incomplete diallel set in moisturized Petri dishes incubated in growth chamber. At 4 d after imbibition, shoot plus root axes were removed from germinating seeds and their dry weights were determined for the 15 entries (100 ~ 200 grains per entry with three independent replicates). Highly significant difference (P < 0.001) in shoot plus root axes dry weight (ADW) from 4-d-old germinating seeds was found among the nine crosses and the six parents using an F-test. The F_1s producing highest dry mass had ‘Zhenshan 97’ and ‘Mianhui 725’ as one or both of the parents (Table 1). It is interesting to note that

the cross Zhenshan 97 × Minghui 63, producing F_1 hybrid named Shanyou 63 which is one of the most widely cultivated rice hybrids in China during the last two decades, demonstrated the highest axes dry mass.

Substantial heterosis was detected for ADW, with MPH varying from 3.7% for the cross Jin 23 × Minghui 63 to 64.4% for the cross Zhenshan 97 × Minghui 63 (Table 1). Thus the crosses showing the lowest heterosis and the one with the highest heterosis both involved Minghui 63. Moreover, in five out of the nine crosses (InDi_09 and InDi_12~15, see Table 1 for the genotypes), there were significant differences between parents and F_1 hybrids via one-way ANOVA analyses ($P < 0.01$), and all of the five crosses exhibited significant high-parent heterosis (F_1 shows better performance than the higher parent) for the character, with the level of high parent heterosis ranging from 30.1% to 49.5%. There was no significant correlation between performance or heterosis for shoot plus root axes dry mass and seedling growth traits.

Levels of Endogenous GAs in Rice Seedlings and Germinating Seeds

GAs have a well-characterized involvement in promoting vegetative growth in rice (Kaneko et al., 2003; Sakamoto et al., 2004). According to our survey and previous studies (Akita et al., 1990; de Leon et al., 2001), heterosis in rice seedling growth could be detected as early as 16 ~ 23 DAS with varying degree. To assay the accumulation levels of GAs, including active forms as well as their metabolites and precursors (Fig. 1), at early stage of rice seedling growth which may be part of the hormonal basis of seedling heterosis, the growing shoots from 20 DAS seedlings and the shoot plus root axes from 4-d-old germinating seeds of the incomplete diallel set of nine crosses were harvested and the endogenous GA levels were analyzed by combined gas chromatography-mass spectrometry (GC-MS).

The concentrations and distribution patterns of 10 quantified GA species, all of which, except GA_4 and GA_{34}, belong to the early-13-hydroxylation pathway (Fig. 1) that is the dominant pathway for GAs in vegetative growth of rice, are shown in the heat map (Fig. 3). The concentrations of GA_{19} and GA_{20} were highest in both tissues, although they varied among the 15 entries. The level of GA_{53} was high in shoots at 20

DAS, but moderate in 4 d germinating seeds, compared to GA_{44} that was present at very high levels in shoots at 20 DAS, but very low levels in 4 d germinating seeds. Low to moderate levels of GA_1, GA_8, GA_3 and GA_4 were detected in both tissues, also with considerable variation among the 15 entries. Finally, GA_{29} and GA_{34} were detected at very low levels in both tissues in all the entries.

The levels for eight of the 10 GA molecules (except for GA_4 and GA_3) were significantly ($P < 0.01$) higher in samples from 20 DAS seedlings than 4-d-old germinating seeds (Fig. 3), which is consistent with the presence of GAs mainly in actively growing and elongating tissues. It is noteworthy that the accumulation of GA_{44}, an intermediate of the reaction in GA biosynthesis pathway catalyzed by GA 20-oxidase (Fig. 1), increased 80 ~ 236 times in the growing shoots of 20 DAS seedlings compared to that in the axes of 4-d-old germinating seeds. By contrast, the contents of GA_{20}, the product of GA 20-oxidase and the immediate precursor for bioactive GA_1 (Fig. 1), did not change much in these two stages although the overall levels were high. In addition, in 20 DAS seedlings, the levels of GA_1 were much higher than GA_4, which is consistent with previous reports that GA_1 acts dominantly during the vegetative stages of rice while GA_4 is the predominant bioactive form in the reproductive organs.

We also displayed the GA contents in the hybrids (H) relative to their parents (MP) (Fig. 4), to illustrate heterosis in GA contents. As shown in Fig. 4, except for GA_4 and GA_{29} in the axes of germinating seeds, heterotic responses of GA contents in F_1 hybrids were low, and with even some negative heterosis observed for the contents of GA_1 and GA_{34} in the axes of germinating seeds.

In the shoots of 20 DAS seedlings, the contents of GA_{53}, GA_{44} GA_{19} and GA_1 were significantly ($P < 0.05$) different among the nine crosses and the six parents. However, in most cases (each of the 10 GA species in the nine crossing combinations), the differences in GA contents between the hybrids and the parents were not significant (Fig. 4) and the values of the hybrids were close to the means of parents. Even in some rare cases where GA contents between the hybrids and the parents were significantly different (Fig. 4), for example GA_{53} in the cross of Jin 23 × Minghui 63

with H/MP ratio of 0.99 (Fig. 4), the contents in the hybrids were still close to the means of the parents, reflected by the H/MP ratios being about 1.0, which implies the additive effect of the parental genotypes. One exception appeared for the contents of GA_{29} in the cross of Zhenshan 97 × Mianhui 725 where GA_{29} in F_1 hybrid (1.08 ± 0.22 ng g^{-1} DW) was significantly ($P < 0.05$) higher than that in both parents (Fig. 3 and Fig. 4). The concentrations of GA_{53}, GA_{44}, GA_1 and GA_8 in this tissue displayed significant positive correlations ($P < 0.05$) with the shoot dry mass, with correlation coefficients of 0.581, 0.661, 0.567 and 0.523, respectively (Supplemental Table S3). This is consistent with recent reports that bioactive GAs are responsible for elongating shoots and biomass production (Eriksson et al., 2000; Biemelt et al., 2004). There is a positive relationship between the content of bioactive GA_1 and GA_8, its physiologically inactive catabolic product, ($r = 0.773$, $P < 0.01$), reflecting the normal metabolism from GA_1 to GA_8 (Fig. 1).

In the shoot plus root axes of 4-d-old germinating seeds, except for GA_{53} and GA_{44}, highly significant differences ($P < 0.05$) in GAs contents were observed among the nine crosses and the six parents. In 36 (40%) of 90 cases (10 GA species in nine crossing combinations), GA levels in the hybrids showed significant differences ($P < 0.05$) compared to their parents, and in 20 (22%) cases, the hybrids had significantly ($P < 0.05$) higher GA levels relative to the high value parents (Fig. 4). There was only one case where GA_1 content in a F_1 hybrid, Maxie × Mianhui 725 (1.24 ± 0.53 ng g^{-1} DW), was significantly ($P < 0.05$) lower than both parents (Fig. 3 and Fig. 4). We also found that while the levels of bioactive GA_1 in the F_1 hybrids were similar to the low value parents (Fig. 3), the levels of GA_8, the deactivated form of GA_1 (Fig. 1), in the hybrids were close to the high value parents (Fig. 3) and the levels of the bioactive GA_4 in the hybrids were much higher than both parents (Fig. 4). As observed for the 20 DAS seedlings, correlations between GA contents and axes dry mass were also found (Supplemental Table S3). The level of GA_{29}, a deactivated product (catalyzed by GA 2-oxidase) of GA_{20} (Fig. 1), which is the immediate precursor for bioactive GA_1, exhibited a significant positive correlation ($r = 0.9$, $P < 0.01$) with the axes dry mass, whereas in contrast, the content of bioactive GA_1 showed a significant negative

correlation (r = -0.554, $P < 0.05$), implying that bioactive GA_1 plays a negative role in the regulation of dry mass production in the germinating seeds. It is reported that GA action suppresses many facets of photomorphogenesis (Alabadi et al., 2004), and therefore suppression of GA accumulation in specific tissues would be necessary to allow photomorphogenic development of young seedlings. This may explain the negative correlation between GA_1 level and axes dry mass in the tissues of the 4-d-old germinating seeds examined here, likely achieved by phytochrome-mediated induction of *GA2ox* expression in response to light (Zhao et al., 2007).

Expression Levels of Gibberellin Metabolism and Signaling Genes

Gibberellin metabolism, including the biosynthesis of bioactive GAs from *trans*-geranylgeranyl diphosphate (GGDP) and their deactivation, can be divided into three stages: (i) the formation of *ent*-kaurene; (ii) the conversion of *ent*-kaurene to GA_{12}; and (iii) the formation and deactivation of bioactive GAs (Fig. 1; Hedden and Phillips, 2000; Yamaguchi, 2008). Eight types of enzymes are required for this process (Fig. 1), namely *ent*-copalyl diphosphate synthase (CPS), *ent*-kaurene synthase (KS), *ent*-kaurene oxidase (KO) and *ent*-kaurenoic acid oxidase (KAO), GA-20 oxidase (GA20ox), GA-3 oxidase (GA3ox), GA-2 oxidase (GA2ox) and CYP714D1 (Elongated Uppermost Internode, EUI; Yamaguchi, 2008). Once bioactive GAs are synthesized, they are perceived by a soluble GA receptor GID1 (Ueguchi et al., 2005) which, upon binding with GA, becomes capable of interacting with the DELLA protein (SLR1 in rice), a negative regulator of GA response (Ikeda et al., 2001). GID2, a F-box protein (Sasaki et al., 2003), forms a SCF^{GID2} complex which functions as a SCF E3 ubitiquitin ligase targeting SLR1 within the GA-GID1-SLR1 complex for degradation, resulting in derepression of GA responses (Hirano et al., 2008, Fig. 1). In addition, the rice *Dwarf 1* (*D1*) gene encoding the α subunit of heterotrimeric G protein (Gα protein) is thought to function as a positive regulator of GA signaling (Ueguchi-Tanaka et al., 2000, Fig. 1). In rice, the enzymes that catalyze the early steps of GA biosynthesis, CPS, KS, KO and KAO, are encoded by single genes, while those catalyze the late steps in the pathway, including GA20ox, GA3ox

and GA2ox, are each encoded by multigene families, each having four, two and ten gene members, respectively (Sakamoto et al., 2004; Lo et al., 2008). As far as GA signaling factors GID1, GID2, SLR1 and D1 are concerned, they are each encoded by single genes.

To reveal the expression profiles of GA metabolism and signaling genes in the F_1 hybrids and their parents in the 3 × 3 incomplete diallel set, total RNAs from growing shoots of 20 DAS seedlings, were extracted and reverse-transcribed for real-time RT-PCR analysis. *GA20ox2* (*SD1*), also known as the "green revolution gene" in rice, has several naturally occurring mutants, one of which designated as *sd1-1* caused by a 383-bp deletion from the genome has been widely used in rice breeding programs. Through PCR amplification using *SD1* specific primers encompassing the 383-bp deletion area, it was confirmed that the *sd1-1* allele was widespread in the parental lines (including Ce 64, Minghui 63 and Mianhui 725) of the incomplete diallel set used in this study. Furthermore, in the RT-PCR analyses using three pairs of specific primers locating within the 5', middle and 3' regions of *SD1* transcript, transcription could be only detected by the primer pairs from the 3' region in the samples tested (data not shown). Therefore, *OsGA20ox2* was excluded from the analysis. Meanwhile, although 10 *OsGA2oxs* have been identified in the rice genome (*OsGA2ox1* ~ *OsGA2ox10*, with *OsGA2ox10* being a pseudo-gene; Lo et al., 2008), only *OsGA2ox1* ~ *OsGA2ox6* are included in the analysis. In addition, because the transcripts of four genes, *OsGA20ox3*, *OsGA3ox1*, *OsGA2ox2* and *OsEUI*, could not be detected in seedling samples (data not shown), they were also excluded from the real-time RT-PCR analysis. Thus, the expression of a total of 16 GA-related genes was eventually assayed by real-time quantitative RT-PCR (Supplemental Table S1).

For the comparative C_T method of relative quantitation to be valid, the efficiency of the target amplification and that of the reference (endogenous control) must be approximately equal. To check if the assays for the 16 GA-related genes fulfilled the criterion, two genes, *OsSLR1* and *OsCPS1*, were chosen randemly for a validation experiement as described in MATERIALS AND METHODS. The absolute value of the slope for these two genes was 0.028 and 0.090, respectively, which passed this test

because both were < 0.1, indicating that the comparative C_T method is valid for our assays.

Except for *OsGA3ox2*, significant differences in gene expression levels among the 15 genotypes from the 3 × 3 incomplete diallel set (Table 3) were detected with $P < 0.01$ ($P < 0.05$ for *OsGA2ox4*). To determine differentially expressed genes between inbred parents or between parental lines and their F1 hybrids, one-way ANOVA of qRT-PCR data within each triad of the nine crosses was carried out for each of the 16 genes. Thirteen out of the 16 (81%) genes were identified as differentially expressed at the level of $P < 0.05$ in at least one of the nine hybrid-parent triads (Table 3). *OsKO2*, *OsGA3ox2* and *OsSLR1* did not display significant differential expression in any of the nine crosses investigated. The modes of gene action for the 13 genes identified as differentially expressed were further investigated in individual crossing combination (Table 3). Differential expression was identified ($P < 0.05$) in 46 (32%) of the 144 cases, of which only two cases showed an expression pattern that was not distinguishable from additivity, while 95% (44 of 46) exhibited non-additive expression patterns (Table 3). The non-additive differential expression in 44 cases was further classified into four distinct modes based on multiple comparisons ($P < 0.05$): 28 exhibited over-dominance, 14 exhibited positive dominance and two exhibited negative dominance, while no under-dominance was detected. Thus the differential expression of these genes in one or more of the nine crosses is usually characterized by up-regulation in the hybrid relative to the mean of the two parents showing predominantly over-dominant gene action, which is consistent with a recent study of microarray analysis of rice hybrids (Zhang et al., 2008) showing that genes involved in biosynthesis of the hormones exhibited a strong over-dominance/under-dominance mode of gene action. When we looked at the expression patterns of the 13 differentially regulated genes in the nine F_1 hybrids, it was demonstrated that a variety of modes of gene action could be seen simultaneously for one given gene in different crosses (Table 3). For example, four distinct modes of gene action, from negative dominance, additivity, positive dominance to over-dominance, could be detected for *OsGA2ox5* in eight of the nine crosses analyzed. Three kinds of modes, negative

dominance, additivity and postive dominance, appeared for *OsGA2ox3*, while two modes, positive dominance and overdominance, were identified at the same time for *OsKAO*, *OsGA20ox1* and *OsGA20ox4*. These findings are consistent with a recent study in maize (Swanson-Wagner et al., 2006) that supports the involvement of multiple modes of gene action in association with heterosis, and also imply that the mode of gene action is regulated by genetic background. On the other hand, the individual hybrid-parent triad having the largest number of differentially transcribed genes is from the cross Jin 23 × Mianhui 725, with 12 genes expressed predominantly in over-dominance in the F_1 hybrid, followed by Maxie × Mianhui 725 and Zhenshan 97 × Minghui 63, having nine and eight differentially expressed genes, respectively. For the cross Jin 23 × Ce 64, only one gene *OsGA2ox3* was differentially regulated with negative dominance in the F_1 hybrid.

Additionally, the ΔC_T values from real time RT-PCR analyses produced by normalizing the respective C_T value of each gene to that of the endogenous reference, *OsActin1*, can be used to compare the expression levels between the genes analyzed, although this sort of comparison may not, in a strict sense, be completely accurate. We used it merely to reflect the relative differences in expression level among the genes when necessary. In rice seedlings of 20 DAS, the GA-related genes analyzed in this study showed varying expression levels. Among the genes in the early steps of GA biosynthesis (Fig. 1), namely *OsCPS1*, *OsKS1*, *OsKO2* and *OsKAO*, the expression level of *OsKS1* was higher than other genes, which is in agreement with the study in *Arabidopsis* (Yamaguchi et al., 1998) showing that *KS* expression in *Arabidopsis* is at much higher levels than that of *CPS*. Between the two members of *OsGA20oxs* examined in this study, *OsGA20ox4* exhibited higher expression level than *OsGA20ox1*. For the five *OsGA2oxs*, *OsGA2ox1* and *OsGA2ox6* expressed at similar levels but higher than other gene members. Interestingly, the transcript abundance for three GA signaling genes (Fig. 1), *OsGID1*, *OsGID2* and *OsD1*, was much higher than GA metabolism genes, with *OsD1* showing the highest expression level among all the genes. It is thought that GAs are present in most vegetative and floral tissues at low concentrations (0.1 ~ 100.0 ng g^{-1} fresh weight) and their biosynthetic enzymes

are similarly low in abundance (Hedden and Phillips, 2000).

Relationship of GA Contents and Expression of GA-Related Genes with Hybrid Performance and Heterosis of Rice Seedlings

In the growing shoots of rice seedlings at 20 DAS, a significant positive correlation between the shoot dry weight and the content of each of four GA species: GA_{53}, GA_{44}, GA_1 and GA_8 was detected, with correlation coefficients of 0.804 ($P < 0.01$), 0.803 ($P < 0.01$), 0.778 ($P < 0.05$) and 0.739 ($P < 0.05$), respectively (Fig. 5 and Supplemental Table S4). Similarly, significant positive correlations ($P < 0.05$) were also identified between heterosis of shoot dry mass and the contents of GAs, including GA_{53} (r = 0.781), GA_{44} (r = 0.745) and GA_1 (r = 0.754), whereas significant negative correlation was detected between heterosis and GA_{20} (r = -0.691), the immediate precursor of bioactive GA_1 (Fig. 6 and Supplemental Table S4). Besides shoot dry mass, positive correlations of heterosis for tiller number of 20 DAS seedlings with the contents of GAs (Supplemental Table S4) were significant at $P < 0.05$ for GA_{53} (r = 0.676), GA_{44} (r = 0.672) and GA_8 (r = 0.684). We also calculated correlation of GA contents in the seedlings at 20 DAS with seedling traits at 30 DAS observed in the paddyfiled. Only GA_4 was found to be significantly correlated with both shoot dry weight (r = 0.809, $P < 0.01$) and heterosis of shoot dry weight (r = 0.716, $P < 0.05$). It should be noted that the above result and interpretation may be limited by the difference in planting conditions of the seedlings used for GA measurements in growth chamber and those for morphological traits in paddyfield.

Similar results were obtained from gene expression analysis. Among the GA metabolic genes examined, the expression levels of *OsCPS1* (r = 0.791) and *OsGA2ox6* (r = 0.720) exhibited significant positive correlations ($P < 0.05$) with the heterosis of shoot dry mass, and those of *OsCPS1* (r = 0.670) and *OsKAO* (r = 0.832) displayed significant positive correlations ($P < 0.05$) with the heterosis for tiller number (Supplemental Table S5). Intriguingly, significant positive correlations ($P < 0.05$) were frequently observed between the heterosis of shoot dry mass and the levels of heterotic expression indicated by the ratios of expression levels of hybrids relative

to those for their parents (*OsCPS1* r = 0.839; *OsKS1* r = 0.718; *OsKO2* r = 0.707; *OsKAO* r = 0.742; *OsGA2ox1* r = 0.828; *OsGA2ox6* r = 0.757) (Fig. 7 and Supplemental Table S6). Moreover, positive regulators in GA signaling, namely *OsGID1*, *OsGID2* and *OsD1*, also exhibited significant positive correlations ($P < 0.05$) with the heterosis for shoot dry mass (Supplemental Table S5 and S6).

In the shoot plus root axes of 4-d-old germinating seeds, a positive correlation was observed between the axes dry mass and the contents of GA_{29} (r = 0.696, $P < 0.05$), while a significant negative correlation was found between the axes dry mass and GA_{19} (r = -0.666, $P < 0.05$) (Supplemental Table S4). GA_{19} is an intermediate in the 20-oxidation steps (Fig. 1) which is often accumulated to a high level compared to other GA precursors during GA biosynthesis in rice (Kobayashi et al., 1988), while GA_{29} is the catabolic product via 2-oxidation of GA_{20} (Fig. 1), the immediate precursor for bioactive GA_1. The same trends (Supplemental Table S4) were also found in terms of the GA_{19} content versus the heterosis of axes dry mass (r = -0.673, $P < 0.05$) and the heterotic content for GA_{29} versus the heterosis of axes dry mass (r = 0.779, $P < 0.05$). These results indicated that GAs may function as a negative regulator for the heterosis regarding the development of germinated seeds of rice.

DISCUSSION

A comprehensive understanding of the biological mechanism for heterosis will clearly benefit from knowledge at three levels: genetic, molecular and physiological. In rice, although many studies have been performed to contribute to a better understanding of the genetic and molecular basis of heterosis (Stuber et al., 1992; Xiao et al., 1995; Hua et al., 2003; Bao et al., 2005; Huang et al., 2006; Zhang et al., 2008; Wei et al., 2009; He et al., 2010; Song et al., 2010), little effort has been made towards understanding its physiological basis. Plant hormones play a vital role in the physiological regulation of plant growth and development, and it was proposed by Rood *et al.* (1988) that GAs provide a phytohormonal basis for heterosis in maize. The study presented here is designed to examine the hormonal basis for heterosis of rice through an in-depth investigation of the physiological role of GAs in the

regulation of heterosis for seedling growth. For this purpose, we collected and analyzed the data regarding endogenous GAs levels together with expression profiles of the genes underlying GA metabolism and signaling in a 3 × 3 incomplete diallel set. It is worthy to note that all the molecules along the GA metabolic pathway (Fig. 1), including not only bioactive GAs but also their precursors and catabolites, are included in the quantification analysis. To our best knowledge, this study provides the most comprehensive coverage of GA molecular species for heterosis analysis in plants.

A systematic survey of heterosis for seedling growth in rice was performed using the 5 × 6 incomplete diallel set from 11 parents comprising elite germplasm for hybrid rice production. Our results (Supplemental Table S2) showed that heterosis for shoot biomass and tiller number could be detected as early as 20 days after sowing, although, at this stage, the extent of heterosis was not that large (highest MPH of 37%) and even moderate negative heterosis could be detected in a few crosses. With the development of rice seedling, obvious and large heterosis for the same traits was observed at 30 DAS, with MPH up to 62% in shoot dry mass for Maxie × Minghui 63 and 73% in tiller number for Xieqingzao × Minghui 86. The extent of heterosis identified in our study is comparable to that from the research by Zhang et al. (2008) where the heterosis for shoot dry mass at 4-leaf-stage (roughly equivalent to 20 DAS) of Liangyoupei 9, a super hybrid rice variety for commercial production in China, and Nipponbare × 93-11, an interspecific hybrid, was 20% and 68%, respectively, while the heterosis for tillering number at 6-leaf-stage (roughly equivalent to 30 DAS) was 32% and 71%, respectively. Our results confirm the widespread occurrence of heterosis at the seedling stage in rice.

In the later steps of GA biosynthesis (Fig. 1), starting from GA_{12} or GA_{53}, there are two parallel pathways leading to bioactive GAs: the early non-13-hydroxylation pathway and the early 13-hydroxylation pathway. It has been verified that the early 13-hydroxylation pathway prevails in vegetative shoots of rice, while the early non-13-hydroxylation pathway occurs predominantly in anther and floral organs (Choi et al., 1995; Hirano et al., 2008). Initially, we intended to quantify GAs in both

pathways and therefore, included deuterium labeled GA internal standards for both 13-hydroxylated (13-OH) and non-13-hydroxylated (13-H) GAs. However, with the exception of GA_4 and GA_{34}, the majority of 13-H GAs (even their internal standards) could not be detected. Comparison of GA profiles between 20 DAS seedlings and 4-d germinating seeds revealed that 13-OH GA levels were significantly more abundant in the former ($P < 0.01$, per *t* test), especially for two C_{20} GAs, GA_{53} and GA_{44} (Fig. 1), levels of which were very low in 4-d germinating seeds (Fig. 3). However, little change was observed on the level of GA_{20} (Fig. 3), the first C_{19} GA in the pathway (Fig. 1), between the two stages analyzed, indicating that the activity of GA 20-oxidase which catalyzes the three steps from GA_{53} to GA_{20} is similar between the two stages. These results suggest that the much lower levels of GA_{53} and GA_{44} in 4 d-old seeds is more likely caused by reduced activity of early GA biosynthetic steps, namely from GGDP to GA_{53} (Fig. 1), or elevated rates of catabolism conferred by the class C_{20} GA2oxs at the point of GA_{53} (Fig. 1), in 4-d germinating seeds.

On the other hand, the contents of GA_4 in 4-d germinating seeds (Fig. 3) increased significantly in the hybrids relative to their parents to levels comparable to or even exceeding those of GA_1, implying higher flux through the early non-13-hydroxylation pathway in the tissue. Although lack of data for 13-H precursors of GA_4 due to technical reason, it seems likely that the GA metabolism in this tissue is reallocated between the two parallel pathways. Considering the sample preparation process that, in order to mimic the real rice seed germination environment in soil, seeds were germinated under appropriate humidity and temperature in the dark for three days prior to harvesting the shoot and root axes under light within one hour, it is similar to the well-known de-etiolation process. It has been shown that the level of bioactive GA_1 decreased to trace amounts within 4 h during de-etiolation of pea seedlings (Ait-Ali et al. 1999; Gil and García-Martinez 2000; Symons and Reid 2003), and the suppression of GA biosynthesis during de-etiolation may occur on the inhibition of CPS activity (Prisic and Peters, 2007), which is in agreement with our inference. In addition, we speculate based on our observation that the reduction of GA_1 content, which is beneficial for photomorphogenesis of etiolated seedlings after irradiation, is

accompanied by an increase in the level of GA_4 which may act to ensure normal development of biological processes other than those related to the establishment of photomorphogenesis. The observation (Supplemental Table S3) that dry mass accumulation in 4-d germinating seeds prepared in this study correlated negatively with GA_1 content (r = -0.555, $P < 0.05$), while positively with GA_4 content (r = 0.662, $P < 0.01$), is in good agreement with this suggestion.

Differential gene expression (Table 3) was detected for 13 out of the 16 GA-related genes analyzed in this study in at least one of the nine hybrid-parent triads from the 3 × 3 incomplete diallel set, and a surprisingly high level of non-additive gene expression was observed, with over half of the cases demonstrating an over-dominance mode of gene expression followed by high-parent dominance, indicating that most of the genes differentially expressed in hybrid-parent triads were up-regulated in the F_1 hybrid. In the previous studies of transcriptomic analyses of heterotic crosses in rice (He et al., 2010; Wei et al., 2009; Zhang et al., 2008), enrichment of differentially expressed genes in some functional categories classified according to Gene Ontology (GO) was clearly observed and was considered to imply the involvement of such functional pathways underlain by the enriched genes in the growth vigor of hybrid. Hence, the enrichment of differentially transcribed genes in GA metabolism and signaling revealed in our study implies that GAs may be involved in the heterosis of rice seedling growth.

Direct evidence supporting a role for endogenous GAs in the regulation of heterosis in rice comes from our correlation analyses of GA contents and GA-related gene expression with the performance and heterosis of rice seedling growth-related traits. As shown in Fig. 6, significant positive correlations between endogenous amounts of several GA species, including bioactive GA_1, the predominant effector for vegetative growth in rice, and the performance or heterosis of shoot dry mass were clearly identified. Interestingly, the content of GA_8, the C-2 hydroxylated inactive GA acting oppositely to bioactive GA_1, also showed positive correlations with the performance (r = 0.739, $P < 0.05$) and heterosis (r = 0.634, just below the critical value of 0.666) of shoot dry mass. This could be explained as following: the deactivation reaction

from GA_1 to GA_8 catalyzed by GA 2-oxidase (Fig. 1) may be not a rate-limiting step of GA metabolic pathway in the shoots of 20 DAS seedlings; GA_1 could be converted to GA_8 without limitation under the normal growing status of the plants in this study. This explanation is supported by the finding that there is a significant positive correlation between the contents of GA_1 and GA_8 (r = 0.773, P < 0.01). Conversely, in terms of GA_{20}, the immediate precursor of GA_1, its content was negatively correlated with the performance (r = -0.643, just beyond the critical value of -0.666) and heterosis (r = -0.691, P < 0.05) of shoot dry mass. This is because, as the very last step in bioactive GA_1 synthesis, 3β-hydroxylation of GA_{20} to GA_1 catalyzed by GA 3-oxidase (Fig. 1) may be a committed step for GA biosynthesis; the flow of GA precursors to bioactive GA_1 might be strictly controlled at this point in the shoots of 20 DAS seedlings so that the endogenous concentration of bioactive GA_1 could be limited within the proper range necessary for normal seedling shoot growth. In rice, GA 3-oxidase is encoded by two genes, *OsGA3ox1* and *OsGA3ox2*. Unlike *OsGA3ox2* which is expressed universally in various organs throughout the rice life cycle, the expression of *OsGA3ox1* is only detected in the tapetum of the anther and the embryo epithelium (Kaneko et al., 2003). Therefore, the existence of only one transcribed GA 3-oxidase gene, *OsGA3ox2*, in vegetative organs of rice pinpoints the importance of the control of its activity and supports our inference. Although no significant correlations were found between GA contents and tiller number of 20 DAS seedlings, significant positive correlations were observed between the contents of the same set of GA species as those for shoot dry mass and the heterosis of tiller number (Data not shown). This difference in the correlations of GA contents with performance in terms of tiller number or the heterosis of the trait, respectively, reinforces our inference pertaining to the role of GAs in association with heterosis for seedling growth.

Additionally, the results from our gene expression analyses demonstrated that there were significant positive correlations between the levels of heterotic expression of GA biosynthetic genes and the heterosis of shoot dry mass (Fig. 7), suggesting the important role of heterotic expression frequently identified for GA related genes in F_1

hybrids caused by non-additive gene expression in the regulation of heterosis. In agreement with the positive role of GA_{53} and GA_{44} in the regulation of heterosis, heterotic expression levels of all four genes involved in early GA biosynthesis, namely *OsCPS1*, *OsKS1*, *OsKO2* and *OsKAO* (Fig. 1), exhibited significantly high correlations with the heterosis of shoot dry mass. Meanwhile, such positive relationships were also detected for *OsGID1*, *OsGID2* and *OsD1* (Fig. 1), three positive regulators for GA signaling, implying that not only GA metabolism but also GA signaling acts synergistically in the regulation of heterosis for rice seedling growth.

In the present study, tiller numbers for young rice seedling were found to exhibit a substantially high degree of heterosis (Table 2). Tillering is an agronomic trait of great importance for grain yield in rice. Rice tillers are formed by shoot branching and this process appears to be regulated by complex interactions of phytohormones, which coordinate factors influenced by genetic, developmental and environmental signals for axillary meristem activity (McSteen P, 2009; Xing and Zhang, 2010). In a simplified model, auxin, strigolactone and GA inhibit shoot branching, while cytokinin functions to promote it (Lo et al., 2008; McSteen P, 2009), although the underlying molecular mechanisms including the crosstalk among these hormones still remain elusive. Therefore, at least for this trait, investigation of the behavior of the hormones other than GA would be necessary for a better understanding of hormonal basis for heterosis. In particular, besides the role in regulation of the number of tillers in rice, cytokinin has also been shown to affect panicle size and spikelet number via control of meristem activity (Ashikari et al., 2005; Kurakawa et al., 2007). Hence, physiological and molecular analyses of diverse endogenous cytokinins and the related genes are necessary in the future for evaluating their potential role in the regulation of heterosis.

Similar to the temporo-spatial feature of gene expression pattern, the concentration and distribution of a certain hormone, such as GA, would be specific to the tissue and stage of development under investigation. However, in most studies concerning the measurement of phytohormones, including the one presented here, samples analyzed

are actually mixtures of tissues containing different cell types and organelles. Moreover, this situation is exacerbated by the extremely low concentrations of plant hormones, which are usually at the nanomolar level. Therefore, more specialized targeted technologies for phytohormone quantification are needed to precisely reflect the hormonal status within a plant. On the other hand, considering that plant hormones act in concert rather than independently to form a regulatory network governing a process of development, the simultaneous determination of multiple hormone concentrations according to the 'omics' based mothodology, integrated with other molecular evidences, like transcriptome analysis of hormone-related genes, would improve our understanding of the phytohormonal basis for heterosis.

In summary, the data presented in this research confirm the widespread occurrence of heterosis at seedling stage in rice. The combined analyses of fluctuations of endogenous GA contents and expression levels of GA related genes in the incomplete diallel set reveal that GAs play a regulatory role in heterosis for rice seedling growth at the physiological level. Larger scale investigations of multiple plant hormones in more developmental stages of rice seedling growth are anticipated to confirm and extend the current findings.

MATERIALS AND METHODS

Plant Materials

Eleven common indica parental lines widely adopted for commercial hybrid rice production during different time periods in Central China were used in this study, including six restorer lines (Ce 64, Mianhui 725, Minghui 63, Minghui 86, Shuhui 881 and 6078) and maintainers of five male-sterile lines (Jin 23, Maxie, Xieqingzao, Zhenshan 97 and II-32). By intermating between these male-sterile and restorer lines (5 × 6), an incomplete diallel set of 30 crosses was obtained, which were previously examined for yield and a number of agronomic traits in replicated field trials (unpublished data). Note that it was the maintainer lines, rather than the male-sterile lines which were only used here for hybrid production with high efficiency, that were grown for all the analyses in this study.

Survey of Rice Seedling Growth

Dry seeds were soaked in water for 48 h at room temperature (25ºC), and then pre-germinated in moisture for 24 h at 37ºC before sowing to the field. The field experiment was conducted under normal agricultural conditions in June and July in the experimental farm of Huazhong Agricultural University, Wuhan, China. Forty-one genotypes including 11 parents and 30 F_1 hybrids of the incomplete diallel set were arranged in a randomized complete block design with three replications. Fifteen seedlings per genotype were grown in one row with four rows in each plot. At 20 and 30 days after sowing (DAS), ten seedlings in the middle of each row were sampled from each plot and measured for the following traits: seedling height, leaf age, tiller number, leaf length/width, shoot fresh weight and shoot dry weight. For the measurement of dry weight, shoots of fresh seedlings were first quickly dried for 2 h at 120ºC and then kept for 48 h at 80ºC in an oven prior to weighing. Relative growth rates (RGR) were calculated as: $RGR=[Ln(M_2)-Ln(M_1)]/(t_2-t_1)$ (Wareing and Phillips, 1981), where M_1 and M_2 are the shoot dry mass at time points t_1 and t_2, respectively.

On the basis of the analysis of the performance and heterosis of rice seedling growth using the 5 × 6 incomplete diallel set, three restorer lines and three maintainer lines were chosen from the original 11 parental lines to intermate with each other (3 × 3) to form an incomplete diallel subset of nine crosses, which were employed for all of the following analyses: rice seed germination, GA quantification and gene expression.

Survey of Rice Seed Germination

Rice seeds were surface-sterilized with 2% NaOCl in water for 30 min, and then rinsed with sterilized distilled water. The sterilized seeds were soaked in distilled water for one day at 37ºC. The resulting germinated seeds were plated out (6 × 6 pattern) in sealed Petri dishes (90 mm diameter) containing two layers of Whatman No. 1 filter paper moistened with 4.5 ml distilled water. Dishes were incubated at 30ºC for three days in darkness. Three independent replicates were set up for the 15 rice lines (9 crosses plus 6 parents) with 100~200 grains for each genotype in each

replicate. Shoot plus root axes were dissected from grains and the separated tissues were plunged directly into liquid N_2 and stored at -80ºC. After freeze-drying, the dry masses of the shoot plus root axes were determined.

GA Quantification

Independent triplicate samples (~0.5 g dry weight) of growing shoots from 20 DAS seedlings and shoot plus root axes from 4-d-old germinating seeds were analyzed for GA content. The samples from 4-d-old germinating seeds were described above. For the samples from 20 DAS seedlings, rice plants were grown in trays in the form of a randomized block design with three replications. Twenty seedlings of each genotype were taken as one sample for each replicate, with four seedlings of each line in each of five trays per block with 60 cells per tray. The experiment was conducted under the controlled environment at Rothamsted Research institute in the UK, where plants were grown in a Gallenkamp 228 cabinet (Sanyo) under a photosynthetic photon flux density of approximately 300 µmol photons m^{-2} s^{-1} with a 14-h light (30ºC)/8-h dark (22ºC) photoperiod and 70% relative humidity and watered regularly. At 20 DAS, growing shoots were harvested from plants directly into liquid N_2 and stored at -80ºC prior to freeze-drying.

Quantification of GAs was performed as described by Coles et al. (1999) with modifications. Briefly, freeze-dried samples were ground to a fine powder using a ball mill, and then extracted by stirring overnight at 4ºC in 80% (v/v) methanol-water (100 ml) containing appropriate amounts of [17-$^2H_2$]GAs as internal standards, and 833 Bq each of the following tritiated GA standards: [1,2-$^3H_2$]GA_1, [1,2-$^3H_2$]GA_4, 16,17-dihydro[15,16,17-$^3H_4$]GA_{19}, and [1,2,3-$^3H_3$]GA_{20}. After filtration, the residue was re-extracted with methanol (100 ml) for 2 h and refiltered. The combined methanol extracts were evaporated almost to dryness under reduced pressure. The residue was resuspended in water, adjusted to pH 8.0 (1 M KOH), and purified by QAE Sephadex A-25 (Pharmacia) anion-exchange column and C_{18} Solid Phase Extraction cartridge (500 mg; ThermoFisher). The dried GAs were methylated twice with excess diazomethane, dissolved in ethyl acetate (1 ml) and partitioned against

water (1 ml). The ethyl acetate phase was passed through a Bond-Elut NH_2 cartridge (100 mg; Varian) that had been preconditioned with ethyl acetate (1 ml). The remaining water phase was partitioned twice more against ethyl acetate, with the organic phases being passed through the NH_2 cartridge. The pooled ethyl acetate phases were evaporated to dryness *in vacuo*, and then the GA methyl esters were resolved by reverse phase HPLC using conditions described previously (Croker et al., 1990). Collected fractions were separately pooled based on the locations of tritiated GAs, and the pooled fractions were analyzed as methyl ester trimethylsilyl ethers on a ThermoFinnigan GCQ mass spectrometer. Samples in *N*-trimethylsilylfluoroacetamide (10 μl) were diluted with dry ethyl acetate (20 μl) and injected (1 μl) into a TR-1 capillary column (30 m × 0.25 mm internal diameter × 0.25 μm film thickness; Thermo Electron) at 50ºC. The split valve (50:1) was opened after 2 min and the temperature increased at 20ºC min^{-1} to 200ºC and then at 4ºC min^{-1} to 300ºC. The instrument was operated in selective ion monitoring mode, with the selected ions for each GA and its $^2H_2$-labeled internal standard as described by Croker et al. (1990). Amounts of endogenous GAs in the original extracts were determined from previously established calibration curves of the peak area ratios for unlabeled and deuterated GAs plotted against varying molar ratios of the two compounds. The same stock solutions of labeled GAs were used for production of the calibration curves.

Real-Time Quantitative RT-PCR Analysis

For expression analysis of GA metabolism and signaling genes, the incomplete diallel set of nine crosses plus their six parental lines were arranged in a paddy field in a randomized complete block design with three replications. Growing shoots of eight seedlings of 20 DAS from each line of each replicate were mixed and harvested directly into liquid N_2 and stored at -80ºC prior to RNA isolation.

Total RNA was extracted from 100 mg tissue using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). First-strand cDNAs were synthesized from DNaseI-treated total RNA using Superscript II reverse transcriptase (Invitrogen) according to the

manufacturer's instructions. Real-time PCR was performed in an optical 96-well plate on an ABI PRISM 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). Each reaction contained the cDNA equivalent of 50 ng total RNA, 12.5 μl of 2 × SYBR Green Master Mix reagent (Applied Biosystems), and 200 nM each of the gene-specific primers in a final volume of 25 μl. The thermal cycle was set as follows: 50ºC for 2 min, 95ºC for 10 min; 45 cycles of 95ºC for 15 s, 60ºC for 30 s, and 72ºC for 45 s; finally 72ºC for 8 min. The specific primers for real-time PCR are listed in Supplemental Table S1, which were designed using Primer Express v.2.0 (Applied Biosystems) software on the basis of CLUSTALW analysis to ensure the specificity of primers among gene families. Reactions were carried out with three technical replicates and the absence of genomic DNA and primer dimers was confirmed by analysis of RT-minus and water control samples and by examination of dissociation curves. The rice *Actin1* gene was used as an endogenous reference to normalize all samples. A mixture of the reverse transcribed cDNA templates from all of the 15 rice samples was prepared to serve as the common calibrator for all samples, and the same pool of calibrator cDNA was used throughout the study to provide for consistency. The comparative C_T method (also known as $2^{-\Delta\Delta C_T}$ method, Livak and Schmittgen, 2001), was applied to calculate the relative amount of target gene expression, which is presented as the quantity normalized to the endogenous reference *OsActin1* and relative to the calibrator.

A validation experiment was carried out according to the user manual from Applied Biosystems to demonstrate that efficiencies of amplifications of target and reference are approximately equal, which is the prerequisite for a valid $\Delta\Delta C_T$ calculation. Briefly, seven serial dilutions of cDNA of the calibrator by 5-fold were amplified by real-time PCR using primers for target gene and *OsActin1*. The ΔC_T value ($C_{T,\,target} - C_{T,\,OsActin1}$) was calculated for each cDNA dilution. A plot of the log cDNA dilution versus ΔC_T was made. A general criterion for passing a validation expriment is that the absolute value of the slope is < 0.1.

Data Processing and Statistical Analysis

Mid-parent heterosis was calculated as: MPH = (F_1 – MP)/MP in %, in which F_1 is the performance of the hybrid and MP is the average performance of the two parents. Absolute mid-parent heterosis value of each cross, calculated as H = F_1 − (P_1 + P_2)/2 (where H is the amount of heterosis, F_1 is the trait measurement of the hybrid, P_1 and P_2 are the measurements of the parents), were used for statistical analyses. For comparisons among the genotypes from the incomplete diallel set and among the F_1 hybrid and its parents within a crossing combination, one-way ANOVA and appropriate multiple comparison were used; for pairwise comparisons between two groups of data, two-sided *t* tests were used. Pearson correlation coefficients were calculated between pairs of data in question.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Table S1. Oligonucleotide primers for the GA metabolism and signaling genes subjected to real time RT-PCR expression analysis.

Supplemental Table S2. Performance and mid-parent-heterosis of rice seedling traits at 20 and 30 DAS for the 5 × 6 incomplete diallel set.

Supplemental Table S3. Correlation coefficients between endogenous GA levels and rice seedling traits.

Supplemental Table S4. Correlation coefficients between endogenous GA levels and performance or heterosis of rice seedling traits of F_1 hybrids.

Supplemental Table S5. Correlation coefficients between expression levels of GA-related genes and heterosis of rice seedling traits.

Supplemental Table S6. Correlation coefficients between extent of heterotic expression of GA-related genes and heterosis of rice seedling traits.

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FIGURE LEGENDS

Figure 1. Schematic diagram of GA metabolism and signaling pathways in rice. Bioactive GAs are shown in black rectangles. For each step in the metabolic pathway, the reaction is highlighted in color. GA_7 (13-nonhydroxy GA_3) is biosynthesized from GA_9 in a similar pathway to the synthesis of GA_3 from GA_{20}, but is not shown in this figure. EUI is a P450 designated as CYP714D1 that preferentially epoxidizes the 16,17-double bond of non-13-hydroxylated GAs, including GA_4, GA_9, and GA_{12}, to deactivate them, and only the reaction on GA_4 is shown in this figure. Bioactive GAs are perceived by the soluble GA receptor GID1 (Ueguchi et al., 2005). SLR1, a DELLA protein, acts as a negative regulator of the GA responses (Ikeda et al., 2001). SLR1 within the GA-GID1-SLR1 complex is degraded through the SCF^{GID2} complex, resulting in GA responses (Sasaki et al., 2003; Hirano et al., 2008). Apart from these signaling factors, the α-subunit of the heterotrimeric G protein (Gα protein) is suggested to function as a positive regulator of GA signaling (Ueguchi-Tanaka et al., 2000). GGDP, geranylgeranyl diphosphate; CPS, *ent*-copalyl diphosphate synthase; KS, *ent*-kaurene synthase; KO, *ent*-kaurene oxidase; KAO, *ent*-kaurenoic acid oxidase; 20ox, GA 20-oxidase; 13ox, GA 13-oxidase; 3ox, GA 3-oxidase; 2ox, GA 2-oxidase; EUI, elongated uppermost internode (CYP714D1).

Figure 2. Distribution of mid-parent-heterosis for the three traits of rice seedling growth in the 5 × 6 incomplete diallel set. The horizontal axis of each chart is the interval of mid-parent-heterosis (MPH) in percentage, and the vertical axis is the number of crosses. (A), (B), and (C) Distribution of mid-parent-heterosis of shoot dry mass per seedling, tiller number per seedling and relative growth rate per seedling, respectively. Averages of three replicates were used for calculation of MPH. SDW_20 DAS and SDW_30 DAS in (A), shoot dry mass of rice seedling at 20 DAS and 30 DAS, respectively; TN_20 DAS and TN_30 DAS in (B), tiller number of rice seedling at 20 DAS and 30 DAS, respectively.

Figure 3. Heat map of distribution of GA species in the 3 × 3 incomplete diallel set.

Growing shoots of rice seedlings at 20 DAS and shoot plus root axes of 4-d-old germinating seeds were harvested, and the endogenous concentrations of the GA species were analyzed by GC-MS. The relative accumulation patterns are shown in the heat map based on the average value for each GA species. Red and blue colors indicate higher and lower concentrations, respectively. The color scale is shown at the bottom. The value in each block is the concentration (average value, $n = 3$) as ng g^{-1} DW. ND, not detected under the quantification limit. InDi_20D_01 ~ InDi_20D_15, growing shoots of 20 DAS seedlings of the parental lines and crosses from the 3 × 3 incomplete diallel set; InDi_4D_01 ~ InDi_4D_15, shoot plus root axes of 4-d-old germinating seeds of the parental lines and crosses from the 3 × 3 incomplete diallel set. The correspondence between the line number and the line identity is the same as in Table 1. * and ** indicate that the GA contents were significantly different between the two tissues by one-sided pairwise *t*-test at $P < 0.05$ and $P < 0.01$, respectively.

Figure 4. Heat map of relative levels of GA species in the F_1 hybrids versus those in the parents of the 3 × 3 incomplete diallel set. The heterotic responses of GA contents in the F_1 hybrids are shown in the heat map for each GA species. Red and blue colors indicate higher and lower heterotic responses, respectively. The color scale is shown at the bottom. The value in each block is the ratio of the GA content in the F_1 hybrid relative to the average of its two parents (H/MP). Other details are the same as in Figure 3. * and ** indicate that the difference among F_1 hybrid and its parents was significant at $P < 0.05$ and $P < 0.01$, respectively.

Figure 5. Correlation between shoot dry weight and GA content of rice seedlings at 20 DAS. The GAs whose concentrations displayed significant correlations ($P < 0.05$) with shoot dry mass of 20 DAS seedlings are shown. The determination coefficient (R^2) is shown for each plot.

Figure 6. Correlation between heterosis of shoot dry weight and GA content of rice seedlings at 20 DAS. The GAs whose concentrations exhibited significant

correlations ($P < 0.05$) with the heterosis of shoot dry mass of 20 DAS seedlings are shown. Absolute mid-parent-heterosis as described in Materials and Methods was used for correlation analyses. The determination coefficient (R^2) is shown for each plot.

Figure 7. Correlations between heterosis of shoot dry mass and level of heterotic expression of GA-related genes. Three genes whose heterotic expression levels demonstrated significant correlations ($P < 0.05$) with the heterosis of shoot dry mass for 20 DAS seedlings are shown in this figure. They are *OsCPS1*, one of the genes involved in the early GA biosynthesis, *OsGA2ox1* from the *GA2ox* gene family, and *OsGID1*, one of positive regulators of GA signaling. The ratio between the expression level of F_1 hybrid and the mean of the corresponding parental lines (H/MP) was $\log_2$ transformed and used in correlation analyses. The *x* axis is the absolute mid-parent-heterosis, as described in Materials and Methods, for shoot dry weight of 20 DAS seedling, and the *y* axis is the level of heterotic gene expression in F_1 hybrid relative to the parents as Log_2 (H/MP). The determination coefficient (R^2) is shown for each plot.

Table 1. Measurements and mid-parent heterosis (MPH) of shoot dry weight of rice seedling at 20 and 30 DAS and shoot plus root axes dry weight of 4-d-old germinating seeds in the 3 × 3 incomplete diallel set. Data shown are the means ± SE from three biological replicates, and for shoot dry weight, the values were measured on a per seedling basis.

Sample name	Genotype	Shoot dry weight of 20 DAS seedling		Shoot dry weight of 30 DAS seedling		Shoot plus root axes dry weight of 4-d germinating seeds	
		Average (g)	MPH (%)	Average (g)	MPH (%)	Average (g)	MPH (%)
InDi_01	Jin 23	0.25 ± 0.038		1.02 ± 0.15		2.30 ± 0.11	
InDi_02	Maxie	0.25 ± 0.067		0.84 ± 0.09		2.73 ± 0.21	
InDi_03	Zhenshan 97	0.23 ± 0.065		0.96 ± 0.004		1.98 ± 0.23	
InDi_04	Ce 64	0.22 ± 0.035		1.02 ± 0.18		2.39 ± 0.34	
InDi_05	Minghui 63	0.28 ± 0.072		0.90 ± 0.16		2.42 ± 0.12	
InDi_06	Mianhui 725	0.19 ± 0.028		0.70 ± 0.14		2.42 ± 0.03	
InDi_07	Jin 23 × Ce 64	0.20 ± 0.009	-17.6	1.01 ± 0.05	-5.3	3.06 ± 0.15	30.6
InDi_08	Maxie × Ce 64	0.17 ± 0.037	-29.8	1.25 ± 0.39	34.0	3.09 ± 0.10	20.5
InDi_09	Zhenshan 97 × Ce 64	0.19 ± 0.011	-14.5	0.93 ± 0.07	3.1	3.52 ± 0.37	61.1
InDi_10	Jin 23 × Minghui 63	0.28 ± 0.078	11.6	1.19 ± 0.42	18.8	2.44 ± 0.21	3.7
InDi_11	Maxie × Minghui 63	0.25 ± 0.031	4.9	1.41 ± 0.36	61.7	3.05 ± 0.08	18.4
InDi_12	Zhenshan 97 × Minghui 63	0.30 ± 0.030	16.4	1.13 ± 0.13	22.0	3.61 ± 0.19	64.4
InDi_13	Jin 23 × Mianhui 725	0.27 ± 0.093	16.4	1.40 ± 0.42	43.1	3.32 ± 0.14	41.0
InDi_14	Maxie × Mianhui 725	0.20 ± 0.039	-10.1	1.19 ± 0.11	41.0	3.55 ± 0.08	38.0
InDi_15	Zhenshan 97 × Mianhui 725	0.22 ± 0.052	0.6	1.28 ± 0.10	53.1	3.37 ± 0.09	53.5

Table 2. Measurements and MPH of tiller number of rice seedling at 20 and 30 DAS and relative growth rate in the 3 × 3 incomplete diallel set.

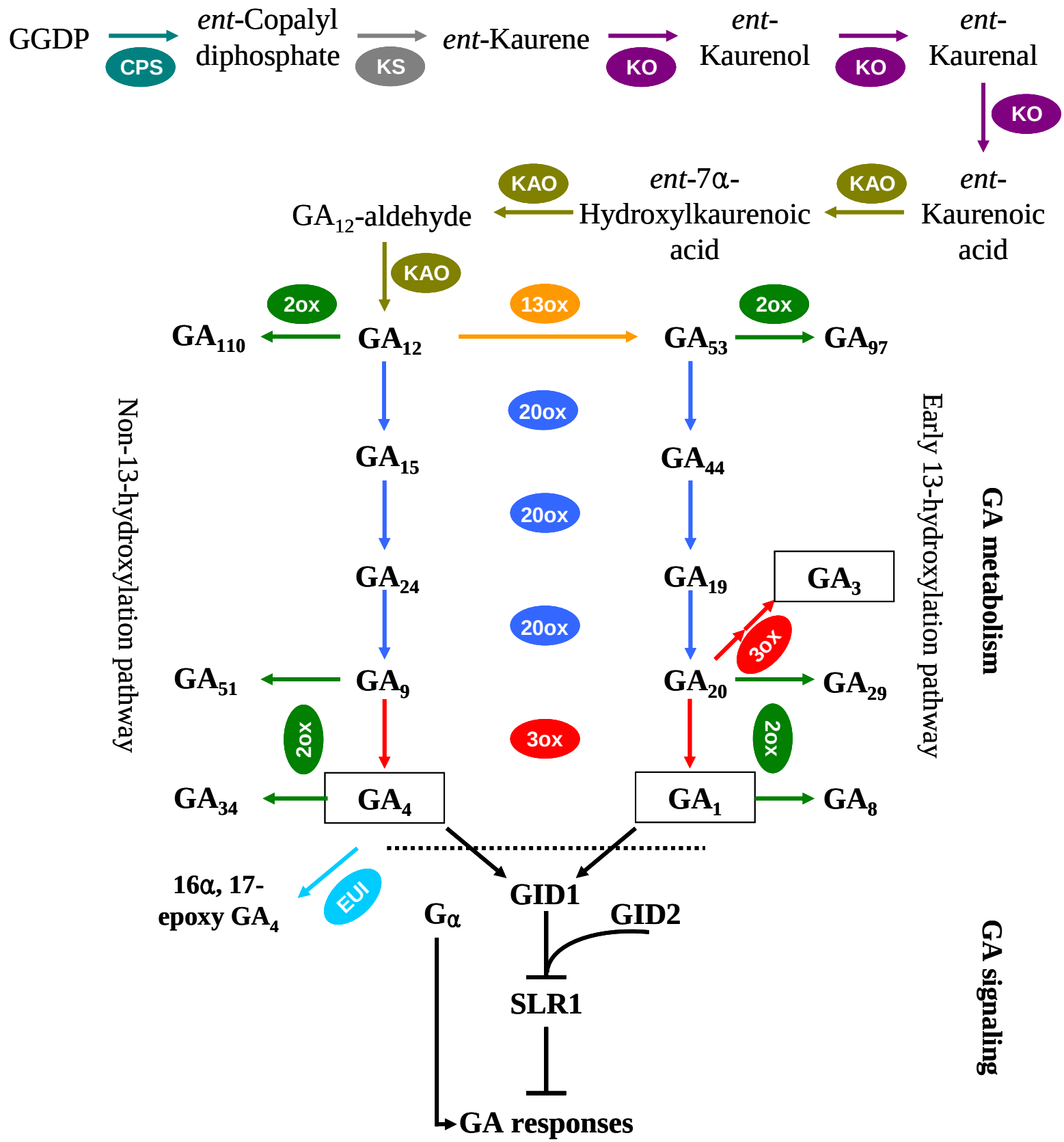
Sample name	Genotype	Tiller number of 20 DAS seedling		Tiller number of 30 DAS seedling		Relative growth rate	
		Average	MPH (%)	Average	MPH (%)	Average (d^{-1})	MPH (%)
InDi_01	Jin 23	2.6 ± 0.1		4.0 ± 0.4		0.14	
InDi_02	Maxie	2.0 ± 0.3		3.8 ± 0.2		0.12	
InDi_03	Zhenshan 97	1.1 ± 0.07		3.8 ± 0.1		0.14	
InDi_04	Ce 64	2.6 ± 0.3		7.5 ± 0.8		0.14	
InDi_05	Minghui 63	1.9 ± 0.4		5.4 ± 0.8		0.12	
InDi_06	Mianhui 725	1.4 ± 0.2		3.4 ± 0.4		0.15	
InDi_07	Jin 23 × Ce 64	2.4 ± 0.06	-7.7	6.1 ± 0.8	6.2	0.16	9.1
InDi_08	Maxie × Ce 64	1.8 ± 0.1	-20.9	7.0 ± 1.0	23.9	0.20	47.4
InDi_09	Zhenshan 97 × Ce 64	2.0 ± 0.3	9.2	5.7 ± 0.6	1.4	0.16	13.5
InDi_10	Jin 23 × Minghui 63	2.1 ± 0.4	-6.0	6.0 ± 1.3	26.6	0.14	4.6
InDi_11	Maxie × Minghui 63	2.2 ± 0.2	14.5	6.9 ± 0.9	50.4	0.17	33.5
InDi_12	Zhenshan 97 × Minghui 63	2.2 ± 0.1	49.3	6.0 ± 0.1	31.7	0.13	3.2
InDi_13	Jin 23 × Mianhui 725	2.1 ± 0.5	4.1	5.4 ± 1.0	47.1	0.16	13.5
InDi_14	Maxie × Mianhui 725	1.5 ± 0.2	-11.5	5.3 ± 0.2	48.8	0.18	32.2
InDi_15	Zhenshan 97 × Mianhui 725	1.7 ± 0.3	32.9	5.0 ± 0.5	40.8	0.18	32.2

Table 3. Heterotic effect of expression levels of GA-related genes in the F_1 hybrids of the 3 × 3 incomplete diallel set in the shoots of 20 DAS rice seedlings. Each value represents a ratio of fold change between F_1 and mid-parent value of gene expression level.

Gene name	InDi_07[a]	InDi_08	InDi_09	InDi_10	InDi_11	InDi_12	InDi_13	InDi_14	InDi_15
*OsCPS1*** [b]	0.929[c]	0.926	1.091	1.365	1.318	1.582 F>M≈P[c]	1.448 F>M≈P	1.478	1.481
*OsKS1***	0.794	0.840	1.138	1.240	1.227	1.550 F>M≈P	1.879 F>M≈P	1.778 F>M≈P	1.425
*OsKO2***	0.921	0.909	1.050	1.400	1.082	1.276	1.375	1.490	1.292
*OsKAO***	1.037	0.925	1.152	1.241	1.426 F>M≈P	1.506 F>M≈P	2.211 F>M≈P	1.631 F>M≈P	1.434 F≈M>P
*OsGA20ox1***	0.869	1.035	1.096	1.183 F≈M>P	1.105	1.076 F≈M>P	1.276 F≈M>P	1.468 F>M>P	1.229 F≈M>P
*OsGA20ox4***	1.243	1.322 F≈P>M	1.245	1.451	1.794 F>M≈P	1.305	2.010 F>M≈P	2.102 F>M≈P	1.596 F≈M>P
OsGA3ox2	0.770	0.969	1.099	1.354	0.959	1.338	1.125	1.686	1.057
*OsGA2ox1***	0.898	0.870	1.008	1.341	1.038	1.217	1.496 F≈P>M	1.220	1.294
*OsGA2ox3***	0.755 F≈P<M	0.983	1.097 F≈MP(M>P)	1.132 F≈M>P	1.248	1.445	1.413	1.509	1.535
*OsGA2ox4**	0.953	1.061	1.228	1.109	1.297	2.153 F>M≈P	1.906 F>M≈P	1.928 F>M≈P	2.142
*OsGA2ox5***	1.228	0.818 F≈P<M	1.489 F≈M>P	1.145 F≈M>P	0.983 F≈MP(M>P)	1.292 F≈M>P	2.710 F>M>P	1.681 F≈M>P	1.408 F≈M>P
*OsGA2ox6***	0.825	0.787	1.286	1.697	1.134	2.232	2.672	2.247	2.007

				F>M≈P		F>M≈P	F>M≈P	F>M≈P	
*OsGID1***	0.854	0.893	0.971	1.270	1.112	1.178	1.507	1.455	1.192
							F>M≈P		
*OsGID2***	0.822	0.915	1.059	1.292	1.188	1.315	1.690	1.706	1.198
							F>M≈P	F>M≈P	
*OsSLR1***	0.870	0.967	1.008	1.219	0.931	1.022	1.390	1.389	1.224
*OsD1***	0.884	1.009	0.941	1.232	1.196	1.477	1.690	1.660	1.385
						F>M≈P	F>M≈P	F>M≈P	F>M≈P

[a]Refer to table 1 for the identity of each genotype. [b]* and ** indicate genes whose expression levels exhibited significant differences among the 15 genotypes at $P < 0.05$ and $P < 0.01$, respectively. For each gene, comparisons of relative gene expression levels among the three genotypes from each of the nine hybrid-parent triads were carried out by one-way ANOVA, and where significant differential gene expression ($P < 0.05$) was identified, the detail mode of gene action is shown. [c]<, > indicate significantly ($P < 0.05$) larger or smaller, while ≈ indicates no significant difference. M, female parent; P, male parent; F, F_1 hybrid. F>M≈P, overdominance; F≈M>P, positive dominance; F≈M>P, additivity; F≈P<M, negative dominance.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CPS
ent-Copalyl diphosphate
KS
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KO
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KAO
ent-7α-Hydroxylkaurenoic acid
KAO
GA12-aldehyde
KAO
GA110
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GA12
13ox
GA53
2ox
GA97
20ox
GA15
GA44
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GA24
GA19
GA3
20ox
30ox
GA51
2ox
GA9
GA20
GA29
3ox
2ox
GA34
GA4
GA1
GA8
16α, 17-epoxy GA4
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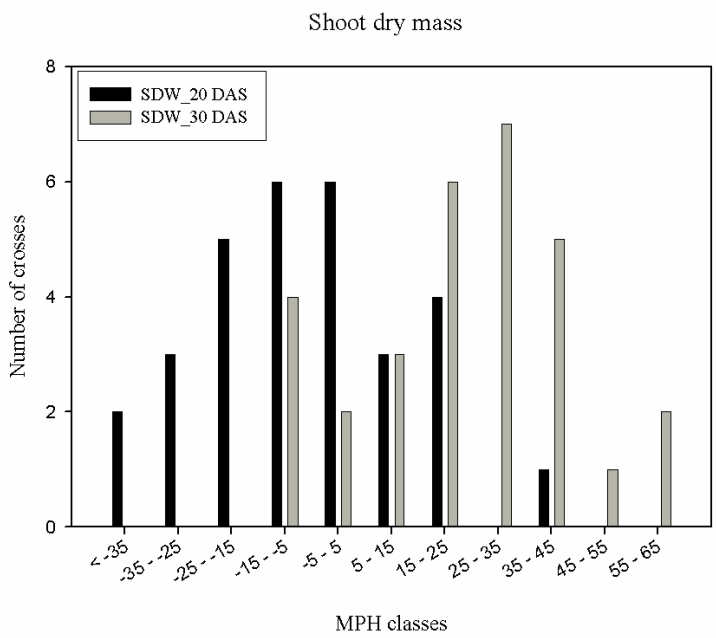
GID1
GID2
SLR1
GA responses
Non-13-hydroxylation pathway
Early 13-hydroxylation pathway
GA metabolism
GA signaling

A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SDW_30 DAS
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4
6
8
< -35
-35 - -25
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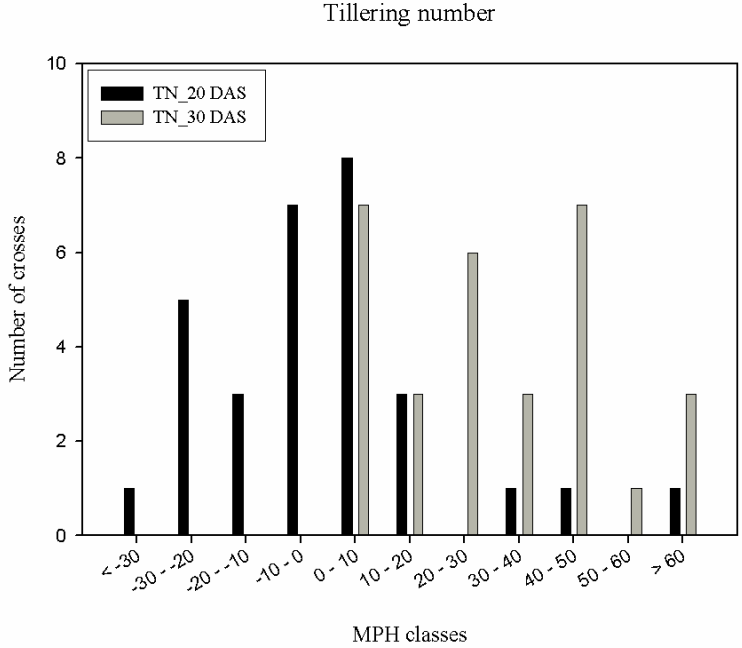
-5 - 5
5 - 15
15 - 25
25 - 35
35 - 45
45 - 55
55 - 65
MPH classes

B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TN_20 DAS
TN_30 DAS
Number of crosses
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2
4
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8
10
< -30
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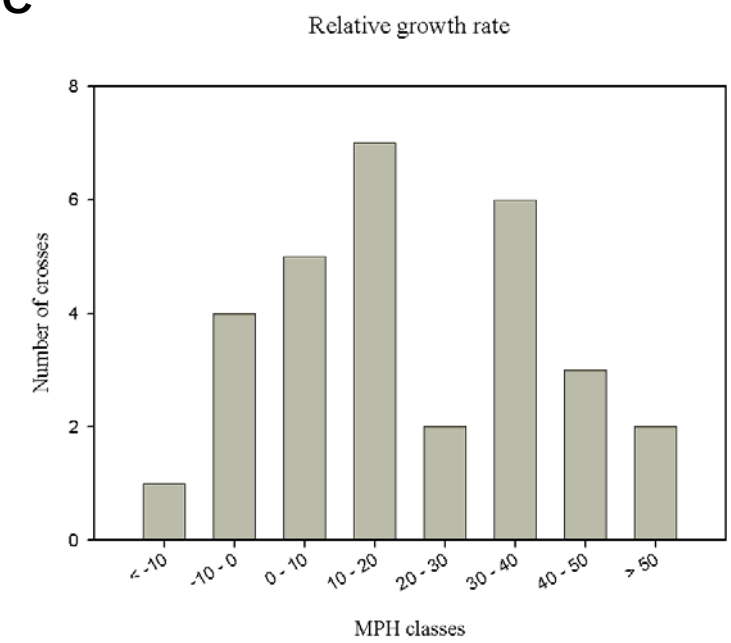
20 - 30
30 - 40
40 - 50
50 - 60
> 60
MPH classes

C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0 - 10
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20 - 30
30 - 40
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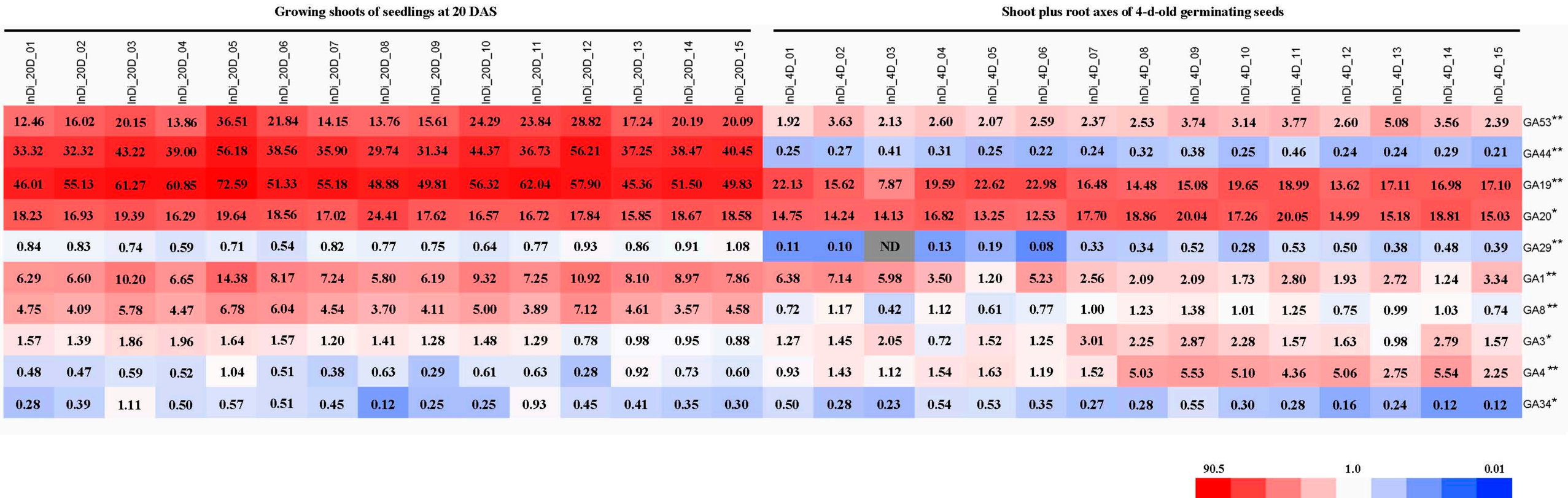
> 50
MPH classes


Growing shoots of seedlings at 20 DAS
Shoot plus root axes of 4-d-old germinating seeds
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InDi_20D_03
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InDi_20D_05
InDi_20D_06
InDi_20D_07
InDi_20D_08
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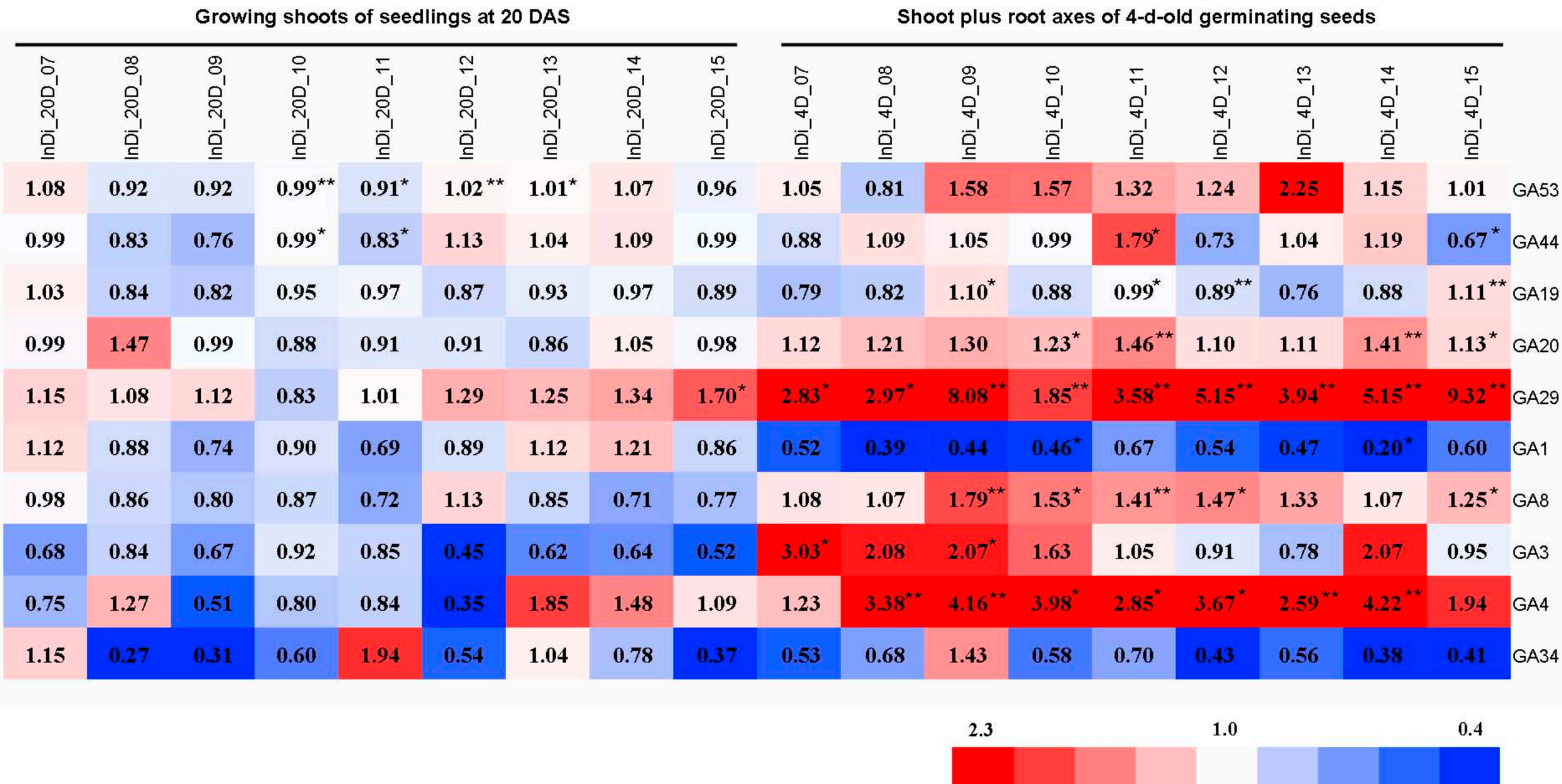
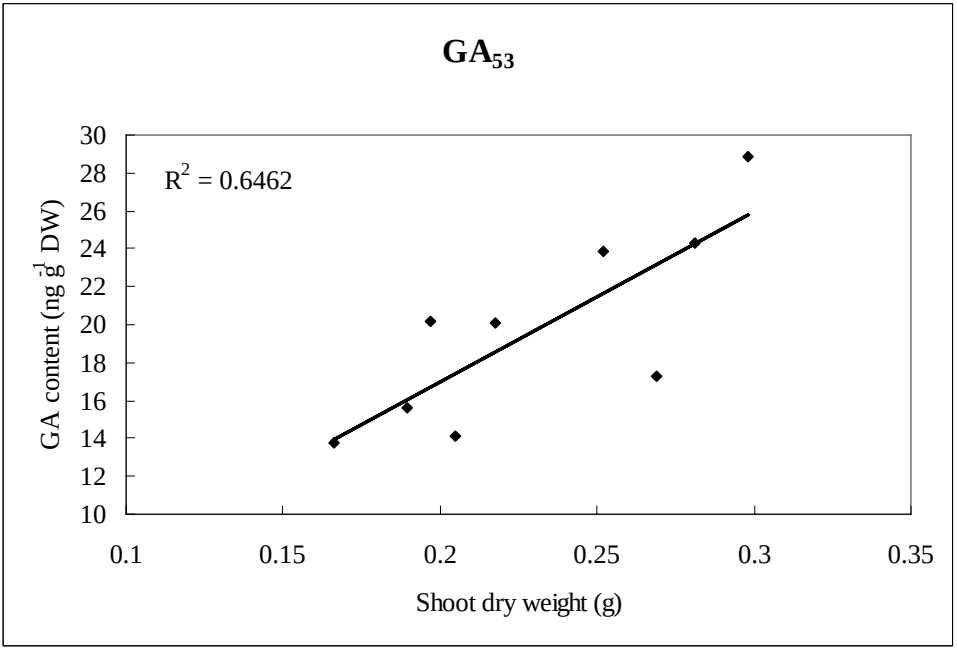
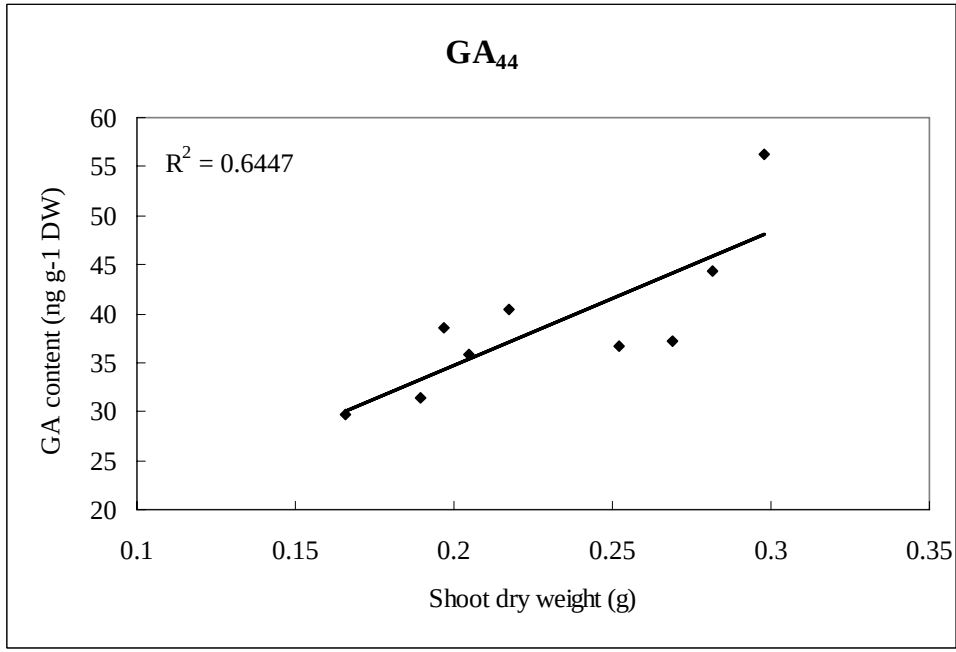
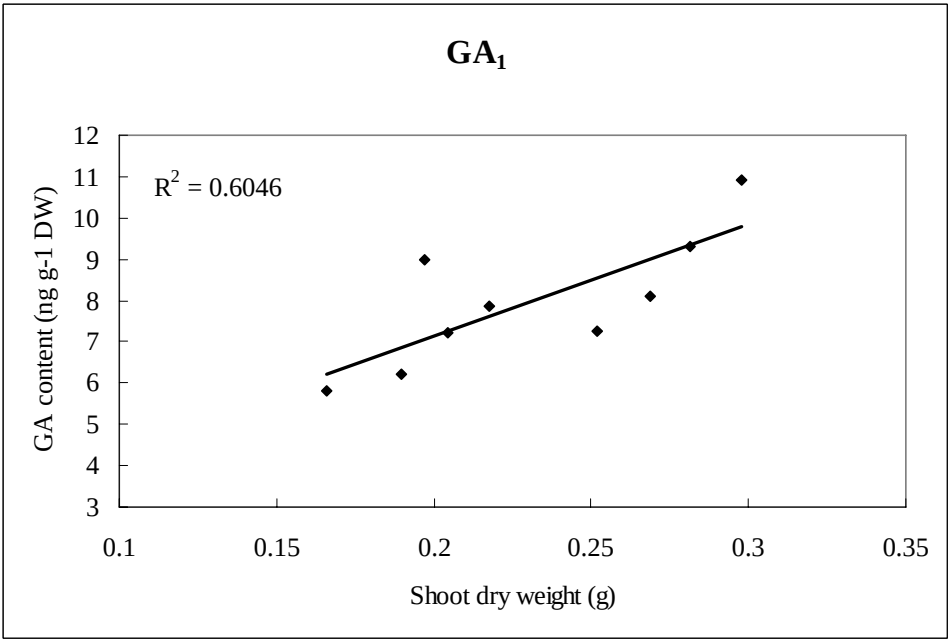
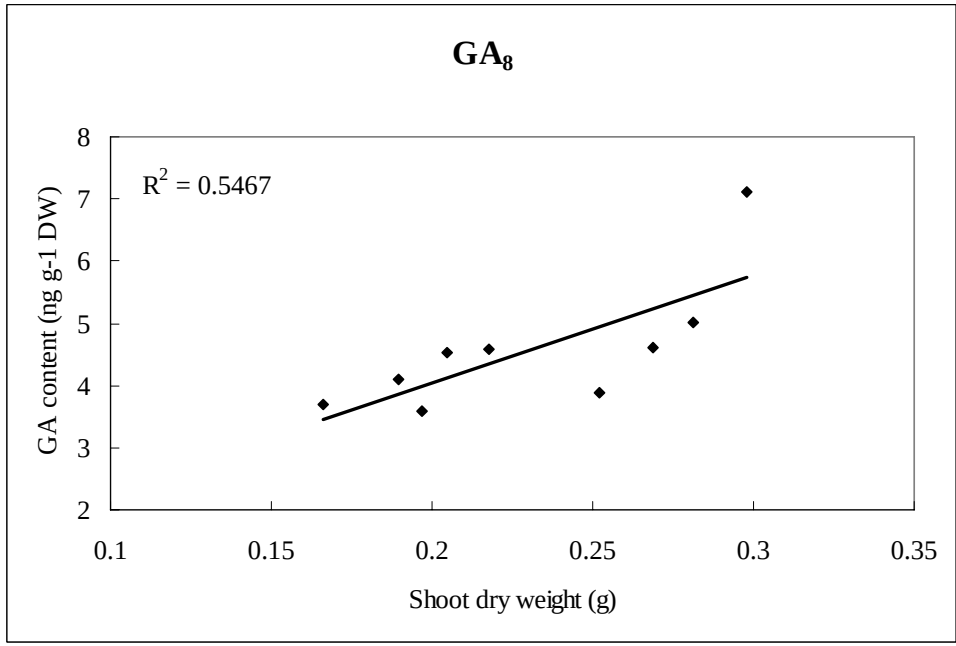
InDi_4D_14
InDi_4D_15
12.46 16.02 20.15 13.86 36.51 21.84 14.15 13.76 15.61 24.29 23.84 28.82 17.24 20.19 20.09 1.92 3.63 2.13 2.60 2.07 2.59 2.37 2.53 3.74 3.14 3.77 2.60 5.08 3.56 2.39 GA53**
33.32 32.32 43.22 39.00 56.18 38.56 35.90 29.74 31.34 44.37 36.73 56.21 37.25 38.47 40.45 0.25 0.27 0.41 0.31 0.25 0.22 0.24 0.32 0.38 0.25 0.46 0.24 0.24 0.29 0.21 GA44**
46.01 55.13 61.27 60.85 72.59 51.33 55.18 48.88 49.81 56.32 62.04 57.90 45.36 51.50 49.83 22.13 15.62 7.87 19.59 22.62 22.98 16.48 14.48 15.08 19.65 18.99 13.62 17.11 16.98 17.10 GA19**
18.23 16.93 19.39 16.29 19.64 18.56 17.02 24.41 17.62 16.57 16.72 17.84 15.85 18.67 18.58 14.75 14.24 14.13 16.82 13.25 12.53 17.70 18.86 20.04 17.26 20.05 14.99 15.18 18.81 15.03 GA20*
0.84 0.83 0.74 0.59 0.71 0.54 0.82 0.77 0.75 0.64 0.77 0.93 0.86 0.91 1.08 0.11 0.10 ND 0.13 0.19 0.08 0.33 0.34 0.52 0.28 0.53 0.50 0.38 0.48 0.39 GA29**
6.29 6.60 10.20 6.65 14.38 8.17 7.24 5.80 6.19 9.32 7.25 10.92 8.10 8.97 7.86 6.38 7.14 5.98 3.50 1.20 5.23 2.56 2.09 2.09 1.73 2.80 1.93 2.72 1.24 3.34 GA1**
4.75 4.09 5.78 4.47 6.78 6.04 4.54 3.70 4.11 5.00 3.89 7.12 4.61 3.57 4.58 0.72 1.17 0.42 1.12 0.61 0.77 1.00 1.23 1.38 1.01 1.25 0.75 0.99 1.03 0.74 GA8**
1.57 1.39 1.86 1.96 1.64 1.57 1.20 1.41 1.28 1.48 1.29 0.78 0.98 0.95 0.88 1.27 1.45 2.05 0.72 1.52 1.25 3.01 2.25 2.87 2.28 1.57 1.63 0.98 2.79 1.57 GA3*
0.48 0.47 0.59 0.52 1.04 0.51 0.38 0.63 0.29 0.61 0.63 0.28 0.92 0.73 0.60 0.93 1.43 1.12 1.54 1.63 1.19 1.52 5.03 5.53 5.10 4.36 5.06 2.75 5.54 2.25 GA4**
0.28 0.39 1.11 0.50 0.57 0.51 0.45 0.12 0.25 0.25 0.93 0.45 0.41 0.35 0.30 0.50 0.28 0.23 0.54 0.53 0.35 0.27 0.28 0.55 0.30 0.28 0.16 0.24 0.12 0.12 GA34*
90.5
1.0
0.01

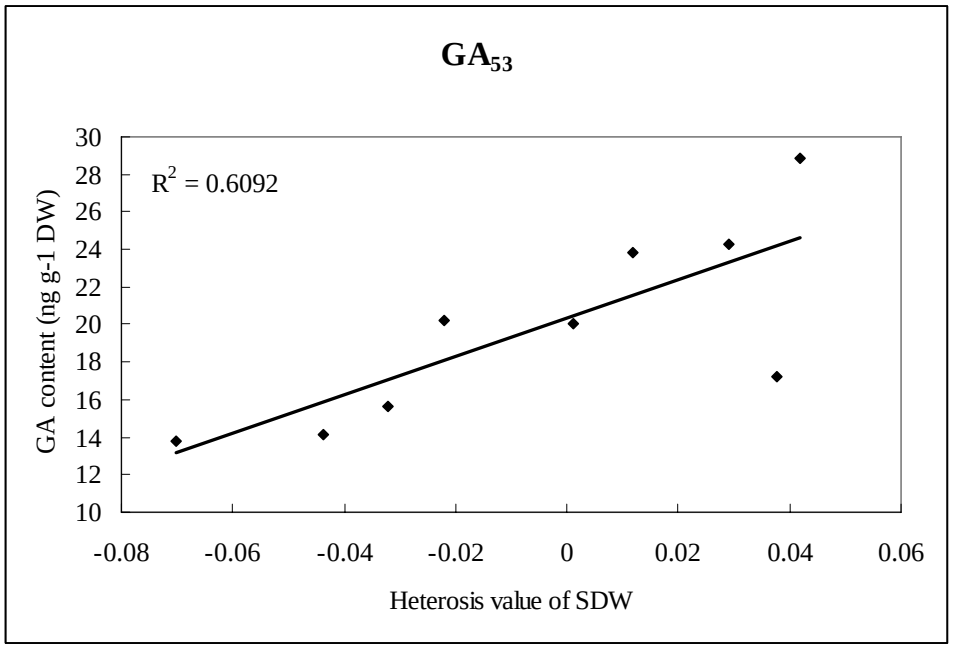

Growing shoots of seedlings at 20 DAS
Shoot plus root axes of 4-d-old germinating seeds
InDi_20D_07
InDi_20D_08
InDi_20D_09
InDi_20D_10
InDi_20D_11
InDi_20D_12
InDi_20D_13
InDi_20D_14
InDi_20D_15
InDi_4D_07
InDi_4D_08
InDi_4D_09
InDi_4D_10
InDi_4D_11
InDi_4D_12
InDi_4D_13
InDi_4D_14
InDi_4D_15
1.08 0.92 0.92 0.99** 0.91* 1.02** 1.01* 1.07 0.96 1.05 0.81 1.58 1.57 1.32 1.24 2.25 1.15 1.01 GA53
0.99 0.83 0.76 0.99* 0.83* 1.13 1.04 1.09 0.99 0.88 1.09 1.05 0.99 1.79* 0.73 1.04 1.19 0.67* GA44
1.03 0.84 0.82 0.95 0.97 0.87 0.93 0.97 0.89 0.79 0.82 1.10* 0.88 0.99* 0.89** 0.76 0.88 1.11** GA19
0.99 1.47 0.99 0.88 0.91 0.91 0.86 1.05 0.98 1.12 1.21 1.30 1.23* 1.46** 1.10 1.11 1.41** 1.13* GA20
1.15 1.08 1.12 0.83 1.01 1.29 1.25 1.34 1.70* 2.83* 2.97* 8.08** 1.85** 3.58** 5.15** 3.94** 5.15** 9.32** GA29
1.12 0.88 0.74 0.90 0.69 0.89 1.12 1.21 0.86 0.52 0.39 0.44 0.46* 0.67 0.54 0.47 0.20* 0.60 GA1
0.98 0.86 0.80 0.87 0.72 1.13 0.85 0.71 0.77 1.08 1.07 1.79** 1.53* 1.41** 1.47* 1.33 1.07 1.25* GA8
0.68 0.84 0.67 0.92 0.85 0.45 0.62 0.64 0.52 3.03* 2.08 2.07* 1.63 1.05 0.91 0.78 2.07 0.95 GA3
0.75 1.27 0.51 0.80 0.84 0.35 1.85 1.48 1.09 1.23 3.38** 4.16** 3.98* 2.85* 3.67* 2.59** 4.22** 1.94 GA4
1.15 0.27 0.31 0.60 1.94 0.54 1.04 0.78 0.37 0.53 0.68 1.43 0.58 0.70 0.43 0.56 0.38 0.41 GA34
2.3
1.0
0.4

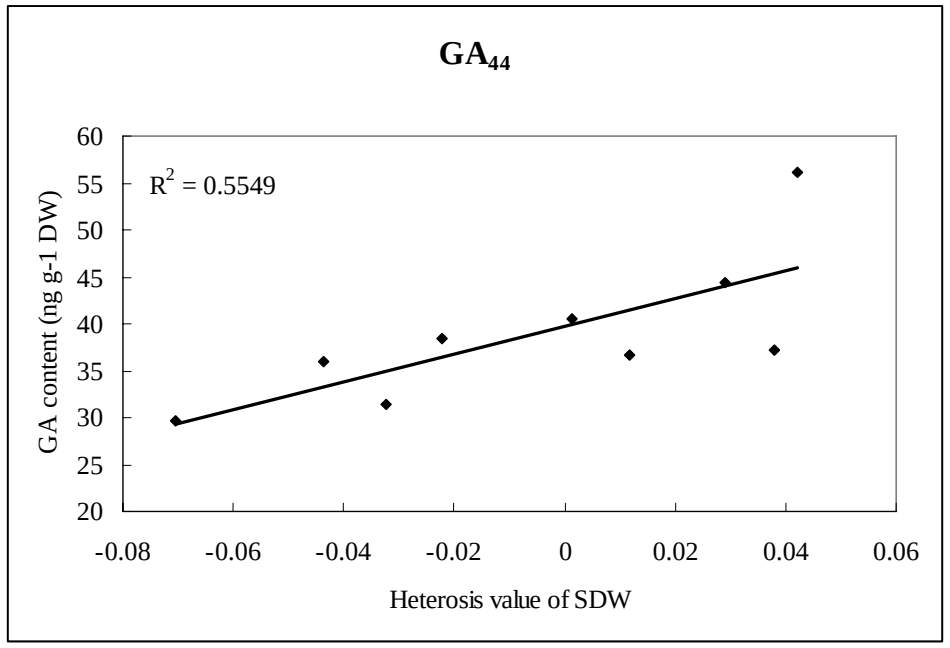

GA53
R2 = 0.6462
GA content (ng g-1 DW)
Shoot dry weight (g)
30
28
26
24
22
20
18
16
14
12
10
0.1
0.15
0.2
0.25
0.3
0.35

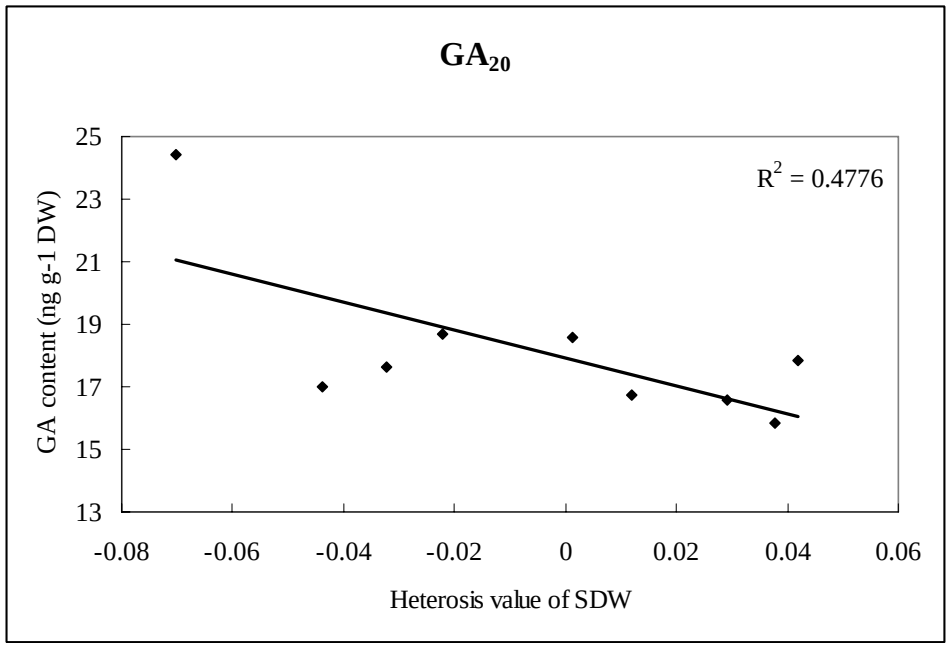

GA44
R2 = 0.6447
GA content (ng g-1 DW)
Shoot dry weight (g)
60
55
50
45
40
35
30
25
20
0.1
0.15
0.2
0.25
0.3
0.35

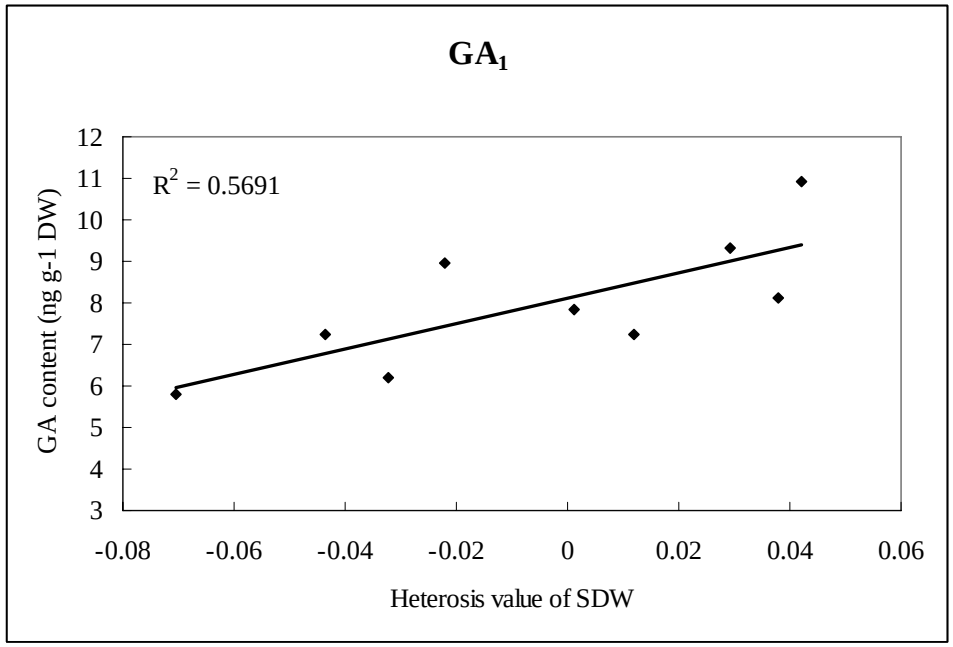

GA1
R2 = 0.6046
GA content (ng g-1 DW)
Shoot dry weight (g)
12
11
10
9
8
7
6
5
4
3
0.1
0.15
0.2
0.25
0.3
0.35

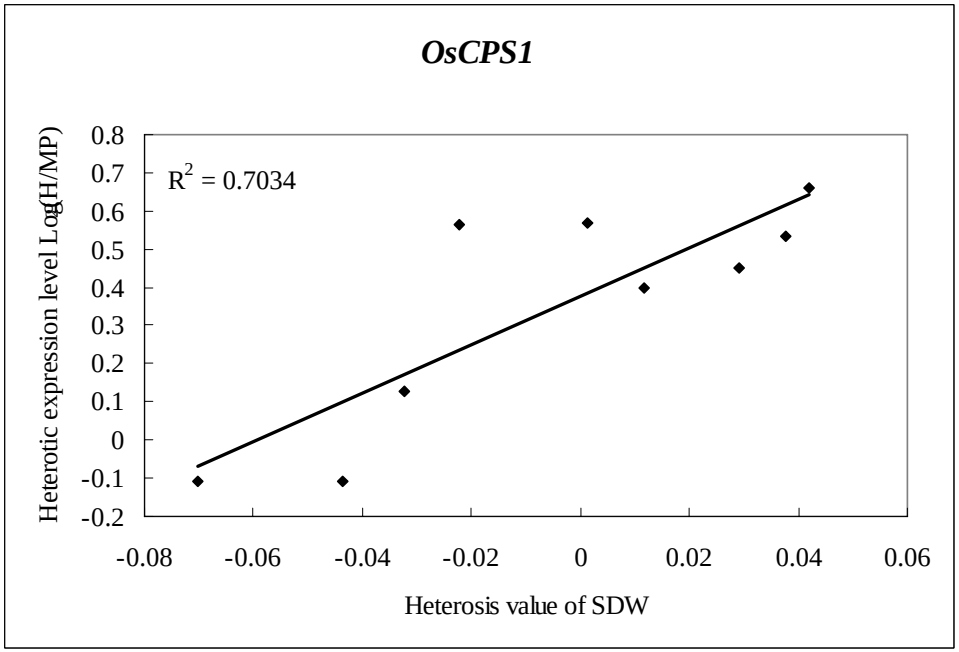

GA8
R2 = 0.5467
GA content (ng g-1 DW)
Shoot dry weight (g)
8
7
6
5
4
3
2
0.1
0.15
0.2
0.25
0.3
0.35

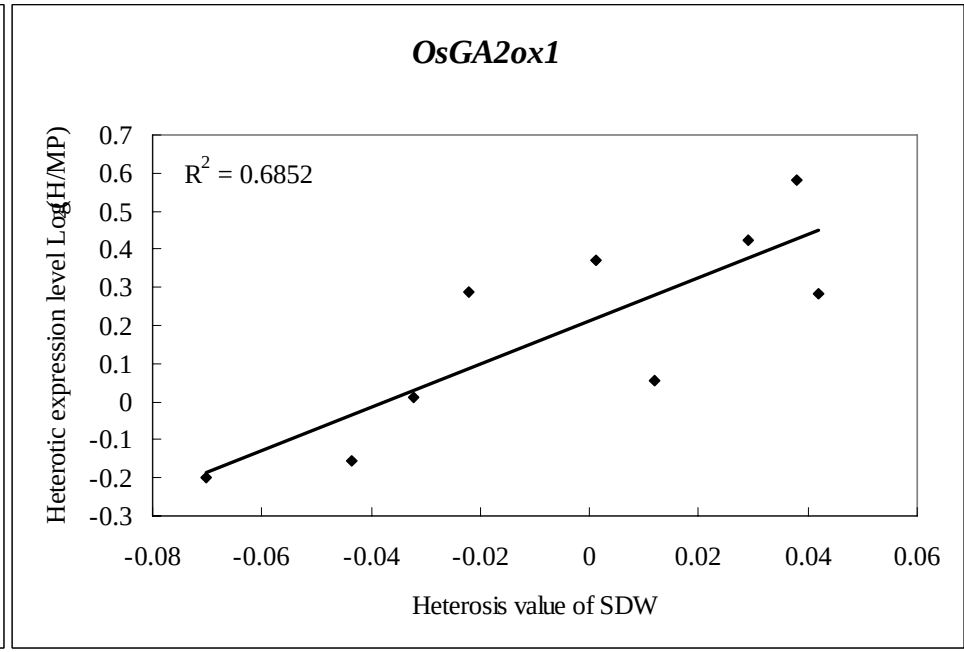

GA53
R2 = 0.6092
GA content (ng g-1 DW)
Heterosis value of SDW

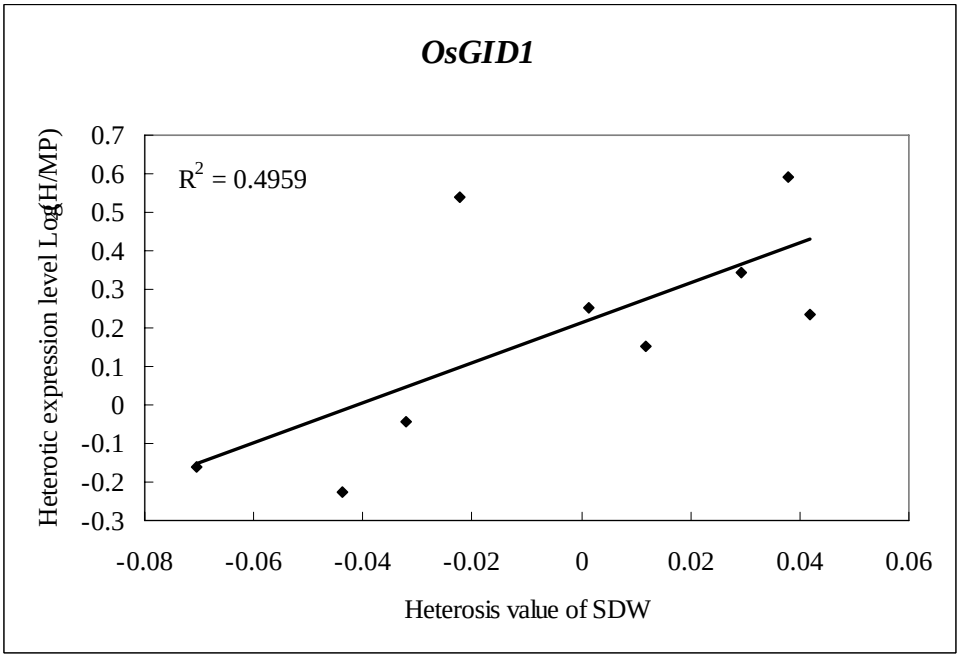

GA44
R2 = 0.5549
GA content (ng g-1 DW)
Heterosis value of SDW


GA20
R2 = 0.4776
GA content (ng g-1 DW)
Heterosis value of SDW


GA1
R2 = 0.5691
GA content (ng g-1 DW)
Heterosis value of SDW


OsCPS1
R2 = 0.7034
Heterotic expression level Log2(H/MP)
Heterosis value of SDW
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.08
-0.06
-0.04
-0.02
0
0.02
0.04
0.06


OsGA2ox1
R2 = 0.6852
Heterotic expression level Log2(H/MP)
Heterosis value of SDW
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.08
-0.06
-0.04
-0.02
0
0.02
0.04
0.06


OsGID1
R2 = 0.4959
Heterotic expression level Log2(H/MP)
Heterosis value of SDW
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.08
-0.06
-0.04
-0.02
0
0.02
0.04
0.06