Methyl Jasmonate Reduces Grain Yield by Mediating Stress Signals to Alter Spikelet Development in Rice[1][W][OA]

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Jasmonic acid (JA) is involved in plant development and the defense response. Transgenic overexpression of the Arabidopsis (*Arabidopsis thaliana*) jasmonic acid carboxyl methyltransferase gene (*AtJMT*) linked to the *Ubi1* promoter increased levels of methyl jasmonate (MeJA) by 6-fold in young panicles. Grain yield was greatly reduced in *Ubi1*:*AtJMT* plants due to a lower numbers of spikelets and lower filling rates than were observed for nontransgenic (NT) controls. *Ubi1*:*AtJMT* plants had altered numbers of spikelet organs, including the lemma/palea, lodicule, anther, and pistil. The loss of grain yield and alteration in spikelet organ numbers were reproduced by treating NT plants with exogenous MeJA, indicating that increased levels of MeJA in *Ubi1*:*AtJMT* panicles inhibited spikelet development. Interestingly, MeJA levels were increased by 19-fold in young NT panicles upon exposure to drought conditions, resulting in a loss of grain yield that was similar to that observed in *Ubi1*:*AtJMT* plants. Levels of abscisic acid (ABA) were increased by 1.9- and 1.4-fold in *Ubi1*:*AtJMT* and drought-treated NT panicles, respectively. The ABA increase in *Ubi1*:*AtJMT* panicles grown in nondrought conditions suggests that MeJA, rather than drought stress, induces ABA biosynthesis under drought conditions. Using microarray and quantitative polymerase chain reaction analyses, we identified seven genes that were regulated in both *Ubi1*:*AtJMT* and drought-treated NT panicles. Two genes, *OsJMT1* and *OsSDR* (for short-chain alcohol dehydrogenase), are involved in MeJA and ABA biosynthesis, respectively, in rice (*Oryza sativa*). Overall, our results suggest that plants produce MeJA during drought stress, which in turn stimulates the production of ABA, together leading to a loss of grain yield.

Rice (*Oryza sativa*), the model system for the study of monocotyledonous plants, is a cereal crop consumed by more than half of the world's population. As such, improvements in grain yield are an important focus of research. Since rice plants grow in a paddy field, they are susceptible to water stress and in particular to drought (Yang et al., 2004, 2007). Approximately 20% of the total worldwide rice growing area is prone to drought (Pandey et al., 2007), and drought is one of the major constraints to rice production worldwide. Although drought conditions can alter the growth and development of rice at any time during its life cycle, drought stress during reproductive growth, but not during vegetative growth, results in a loss of grain yield.

Immediately following the transition of rice plants to the reproductive phase, the vegetative meristem is converted into the panicle meristem. The panicle meristem subsequently differentiates in an orderly fashion into primary branches, secondary branches, and spikelet meristems (Ikeda et al., 2004). Rice grain yield is determined by four parameters: number of panicles per plant, number of spikelets per panicle, filling rate, and total seed weight. The number of panicles and spikelets is determined soon after formation of the panicle and spikelet meristems (Sakamoto and Matsuoka, 2008). Drought exposure during the earlier stages of this transition affects the first two parameters more than the other parameters, while drought exposure at later stages of reproductive development affects the filling rate more than the other parameters. The total seed weight is the combined result of the other three parameters. The development of the panicle and/or spikelet meristem is repressed under the drought conditions, resulting in a reduction in the number

[1] This work was supported by the Ministry of Education, Science, and Technology, Korea, through the Crop Functional Genomics Center (grant no. CG2111 to J.-K.K.), by the Biogreen21 Program (grant to J.-K.K.), and by the Korea Science and Engineering Foundation through the Plant Metabolism Research Center at Kyung-Hee University (grant to J.-K.K.).

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www.plantphysiol.org/cgi/doi/10.1104/pp.108.134684

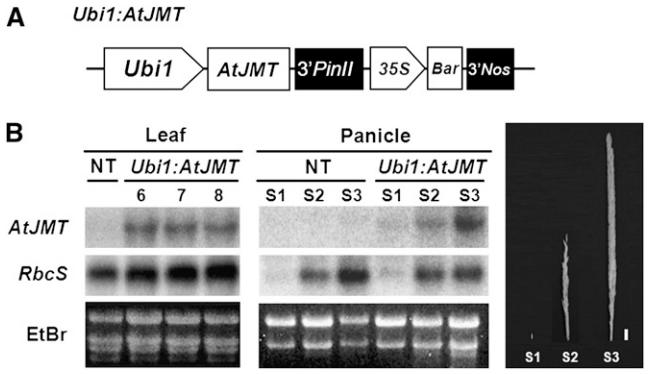

Figure 1. Production of *Ubi1*:*AtJMT* transgenic rice plants. A, *Ubi1*:*AtJMT* consists of the maize *ubiquitin1* promoter (*Ubi1*) linked to the *AtJMT* coding region from Arabidopsis (AY008434; Seo et al., 2001), the 3′ region of the potato (*Solanum tuberosum*) *proteinase inhibitor II* gene (3′*PinII*), and a *bar* expression cassette containing the *35S* promoter of the *Cauliflower mosaic virus*, the *bar* coding region, and the 3′ region of the *nopaline synthase* gene (3′*Nos*). B, RNA gel-blot analysis was performed using total RNAs from the leaves of 4-week-old plants from three independent transgenic lines (lanes 6, 7, and 8) and panicles from *Ubi1*:*AtJMT* and NT plants at three developmental stages. The blots were hybridized with the *AtJMT* probe and reprobed with the rice *RbcS* gene (Kyozuka et al., 1993). *RbcS* transcript levels in the transgenic leaves and panicles were similar to levels in the NT controls. Ethidium bromide (EtBr) staining of total RNA was performed for verification of equal RNA loading. S1 panicles are less than 1.5 cm in length. S2 panicles are not fully elongated (3–10 cm) and are pale white/yellow. S3 panicles are fully elongated and enclosed within the flag leaf sheath during the booting stage. Bar = 1 cm.

of panicles per plant and/or the number of spikelets per panicle (O'Toole and Namuco, 1983; Boonjung and Fukai, 1996; Wopereis et al., 1996; Asch et al., 2005).

Abscisic acid (ABA) has been implicated in a reduction of grain yield following water stress during reproductive plant development. ABA levels were increased upon exposure of plants to drought conditions, which reduces the filling rate by increasing sterility in cereal plants (Morgan, 1980; Ober et al., 1991; Beltrano et al., 1999; Yang et al., 2004, 2007). These studies focused on plant hormone levels during the late stages of reproductive development, including meiosis and/or grain-filling periods following exposure to drought conditions. However, studies on plant hormone levels during the early stages of reproductive development, which determine the number of panicles and spikelets, are currently lacking.

Methyl jasmonate (MeJA) and jasmonic acid (JA) are important cellular regulators involved in diverse plant developmental processes, including seed germination (Nojavan-Asghari and Ishizawa, 1998), callus growth (Ueda and Kato, 1982), primary root growth (Staswick et al., 1992), flowering (Albrechtová and Ullmann, 1994), formation of gum and bulb (Saniewski et al., 1998), and senescence (Ueda and Kato, 1980). They are also involved in plant defense responses to insect wounding, attack by various pathogens, and water deficit (Creelman and Mullet, 1995; Wasternack and Parthier, 1997; Seo et al., 2001; Rakwal et al., 2002). The levels of endogenous jasmonates were reported to be increased following pathogen exposure (Reymond and Farmer, 1998; Thomma et al., 1998). Likewise, exogenous application of jasmonates to plants induced stress-related or pathogenesis-related (PR) genes (Moons et al., 1997; Mei et al., 2006). Thus, the role(s) of jasmonate in response to biotic stresses has been well documented; however, relatively little is known about its involvement in response to abiotic stress. Jasmonate levels were increased in soybean (*Glycine max*; Creelman and Mullet, 1995) and *Pinus pinaster* (Pedranzani et al., 2007) upon plant exposure to drought and in tomato (*Solanum lycopersicum*; Pedranzani et al., 2003) and *Iris hexagona* (Wang et al., 2001) upon exposure to high salinity. In rice, both drought and high salinity increased jasmonate levels in the leaves and roots, resulting in the induction of stress-related PR and JA biosynthetic genes (Moons et al., 1997; Kiribuchi et al., 2005; Tani et al., 2008). These abiotic stress-induced increases in jasmonate levels were observed only in vegetative tissues. Whether drought conditions increase jasmonate levels in reproductive organs remains to be determined.

The jasmonic acid carboxyl methyltransferase (JMT) enzyme converts JA to a volatile component, MeJA. Expression of endogenous *JMT* was not detected in young seedlings, but its expression was initiated in conjunction with the nectar in the developing flowers of Chinese cabbage (*Brassica campestris pekinensis*; Song et al., 2000). Transgenic overexpression of the Arabidopsis (*Arabidopsis thaliana*) JMT gene (*AtJMT*) in Arabidopsis plants using the *35S* promoter increased leaf MeJA levels by 3-fold (Seo et al., 2001). This overexpression also caused a significant decline in transgenic Arabidopsis seed production (Cipollini, 2007). In this study, we attempted to show that MeJA plays an important role in stress-induced loss of grain yield. Transgenic overexpression of *AtJMT* in rice resulted in a large reduction in grain yield through increased MeJA and ABA levels in young panicles. Exposure of nontransgenic plants to drought conditions also increased MeJA and ABA levels in young panicles and significantly reduced grain yield, in a similar manner to that observed in *AtJMT* plants. These results suggest a role for MeJA in the plant response to drought stress.

Table I. *Levels of MeJA and ABA in S1 panicles of NT, Ubi1:AtJMT, and drought-treated NT plants*

The values (ng g^{-1} fresh weight) represent means ± SD of three independent experiments.

Compound	NT	*Ubi1:AtJMT*	Drought-Treated NT
MeJA	222.0 ± 67.5	1,401.8 ± 11.2	4,176.8 ± 223.8
ABA	24.8 ± 2.6	46.3 ± 3.9	34.5 ± 2.2

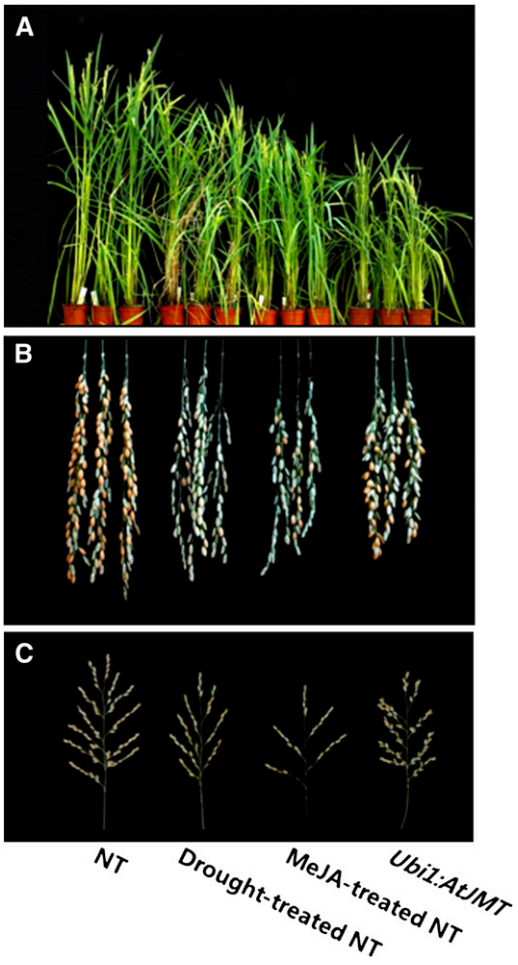

Figure 2. Phenotypes of NT, drought-treated NT, MeJA-treated NT, and *Ubi1*:*AtJMT* plants. A, Plant phenotypes at the grain-filling stage. B, Panicles taken from plants that had reached maturity with ripened grains. C, Panicle architecture.

RESULTS

Ubi1:*AtJMT* Transgenic Rice That Produce High Levels of MeJA in Their Panicles

To study the role of MeJA in reproductive development of rice panicles, rice were transformed with the construct *Ubi1*:*AtJMT* (Fig. 1A), in which *AtJMT* was under the control of the maize (*Zea mays*) *ubiquitin1* promoter, including its first intron (*Ubi1*; Christensen and Quail, 1996). Twelve independent transgenic lines were produced using the *Agrobacterium tumefaciens*-mediated transformation method (Hiei et al., 1994). Of the $T_{1\text{-}6}$ seeds collected, $T_{4\text{-}6}$ homozygous seeds were used for further analysis. Since phenotype was similarly observed in more than three independent transgenic lines, we chose one for extensive study. RNA gel-blot analysis showed that *AtJMT* was expressed in the leaves and developing panicles of *Ubi1*:*AtJMT* plants (Fig. 1B). In the panicles, *AtJMT* expression increased with development from the 1.5-cm-long young panicle (S1) to the fully developed panicle just before the emergence stage (S3; Fig. 1B). Such an increase in expression levels of *AtJMT* appears to be due to different activities of the *Ubi1* promoter in developing panicles (Cornejo et al., 1993). To our knowledge, there is no previous report that directly describes the activity of the *Ubi1* promoter in panicles. MeJA levels were assessed in S1 panicles from transgenic plants and nontransgenic (NT) controls (Table I). *Ubi1*:*AtJMT* panicles had MeJA levels that were 6-fold higher than those of NT panicles. Grain yield, number of spikelets per panicle, and filling rate were also largely reduced in *Ubi1*:*AtJMT* plants as compared with NT controls, a typical phenomenon observed in plants exposed to drought stress. These observations led us to investigate whether MeJA levels were increased by drought stress during reproductive development. Since similar phenotypes were observed in more than three independent transgenic lines, we chose one for further study. NT plants were treated with drought stress at the panicle initiation stage (approximately 30–35 d before heading), and levels of MeJA were measured in S1 panicles. MeJA was 19-fold higher in drought-treated NT panicles than in untreated NT panicles. Levels of ABA, another plant stress hormone, were increased by 1.4-fold in the

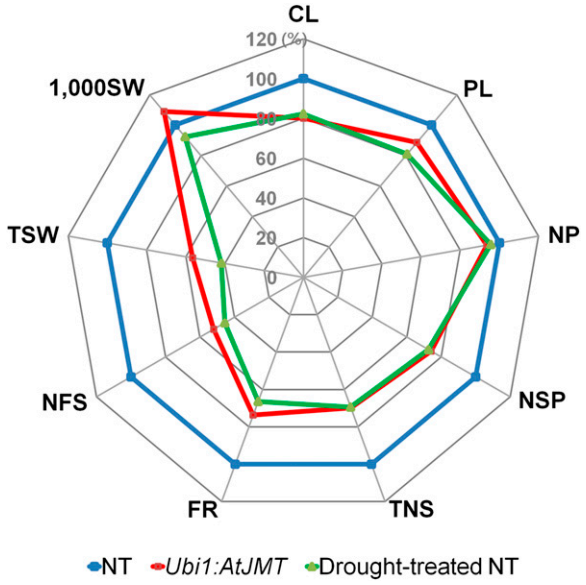

Figure 3. Comparison of agronomic traits between *Ubi1*:*AtJMT* and drought-treated NT plants. A spider plot of *Ubi1*:*AtJMT* plant (red) and drought-treated NT plant (green) agronomic traits as compared with untreated NT plants (blue). Each data point represents a percentage of the mean values ($n = 15$) listed in Table II (summer). Mean values from untreated NT plants were set at 100% as a reference. CL, Culm length; PL, panicle length; NP, number of panicles per plant; NSP, number of spikelets per panicle; TNS, total number of grains; FR, filling rate; NFS, number of filled seeds; TSW, total seed weight; 1,000SW, 1,000 seed weight.

Table II. *Analysis of seed production parameters in NT, Ubi1:AtJMT, and drought-treated and MeJA-treated NT plants*

Each parameter represents the mean ± SD (n = 15 or 30) for NT, *Ubi1*:*AtJMT*, MeJA-treated NT, and drought-treated NT plants. The percentage differences (%Δ) between the values for NT and the values for *Ubi1*:*AtJMT*, MeJA-treated NT, and drought-treated NT was calculated. *P* values were determined by Student's *t* test.

Plants	No. of Panicles	Spikelets per Panicle	Filling Rate	Total Seed Weight	1,000 Seed Weight
	count	*count*	*%*	*g*	*g*
2007 (summer; n = 15)					
NT	23.4 ± 5.2	86.1 ± 6.8	91.2 ± 2.9	42.3 ± 5.4	23.4 ± 0.3
Ubi1:*AtJMT*	21.9 ± 4.2	63.9 ± 7.4	67.2 ± 10.1	23.9 ± 7.5	25.5 ± 3.2
%Δ	−6.6	−25.8	−26.3	−43.5	8.9
P	0.5837	0.0004	0.0005	0.0009	0.1896
Drought-treated NT	22.4 ± 2.9	62.5 ± 8.0	60.5 ± 18.0	17.7 ± 1.8	21.7 ± 1.0
%Δ	−4.3	−27.4	−33.7	−58.1	−7.3
P	0.6997	0.0009	0.0904	0.0023	0.0046
2007 (winter; n = 30)					
NT	7.0 ± 1.7	76.4 ± 13.5	45.6 ± 13.9	5.2 ± 2.2	21.4 ± 1.7
Ubi1:*AtJMT*	5.4 ± 2.2	45.2 ± 10.8	11.8 ± 7.6	0.7 ± 0.7	22.3 ± 2.5
%Δ	−22.28	−40.9	−74.1	−86.0	4.2
P	0.0098	0.0000	0.0335	0.0000	0.1668
MeJA-treated NT	7.3 ± 2.1	31.0 ± 6.8	11.3 ± 13.5	0.6 ± 0.7	22.7 ± 4.9
%Δ	5.0	−59.4	−75.2	−89.3	6.1
P	0.8743	0.0000	0.0000	0.0000	0.1825
Drought-treated NT	6.9 ± 1.5	56.9 ± 11.2	28.3 ± 20.3	2.6 ± 2.3	21.7 ± 2.2
%Δ	−1.0	−25.6	−38.5	−48.8	1.3
P	0.5112	0.00000	0.0003	0.0001	0.5782

drought-treated NT panicles as compared with untreated NT panicles. ABA levels were 1.9-fold higher in S1 panicles from *Ubi1*:*AtJMT* plants than in S1 panicles from untreated NT control plants under non-drought conditions, suggesting that ABA levels were increased by MeJA rather than by drought stress.

High Levels of MeJA Reduce Grain Yield in Both *Ubi1*:*AtJMT* and Drought-Treated NT Plants

Phenotypic evaluation of *Ubi1*:*AtJMT* and NT plants revealed no major differences in the vegetative growth of the entire plants and the time to flowering, although the transgenic plants were slightly smaller than the NT controls. However, grain yield was significantly reduced in the *Ubi1*:*AtJMT* plants (Fig. 2). A similar reduction in plant height and grain yield was also observed in NT plants following drought stress or treatment with exogenous MeJA at the panicle initiation stage (Fig. 2). These observations prompted us to investigate yield components of the *Ubi1*:*AtJMT* and NT plants treated either with drought stress or with exogenous MeJA (Fig. 3; Table II). When compared with untreated NT controls, decreases in the yield parameters of *Ubi1*:*AtJMT* plants were strikingly similar to those observed in drought-treated NT plants (Fig. 3). These experiments were performed twice in the summer and again in the winter of 2007 in a greenhouse, as shown in Table II, obtaining comparable results. Similar reductions in yield parameters were observed in NT plants that were treated with exogenous MeJA, suggesting that MeJA is involved in the loss of grain yield. Specifically, in *Ubi1*:*AtJMT*, MeJA-treated NT, and drought-treated NT plants, the number of spikelets per panicle was reduced by 40.9%, 59.4%, and 25.6%, respectively, and filling rates were reduced by 74.1%, 75.2%, and 38.5%, respectively, as compared with untreated NT control plants. In contrast, 1,000 seed weight of experimental plants remained unchanged. Together, these results suggested that MeJA reduces grain yield by affecting the development of spikelets.

High MeJA Levels Alter Spikelet Organ Numbers in Both *Ubi1*:*AtJMT* and Drought-Treated NT Plants

Several alterations in spikelet organ numbers were noted in the developing spikelets of *Ubi1*:*AtJMT* plants (Fig. 4). NT spikelets are normally composed of a pair of glumes at the base and four whorls of spikelet organs, a lemma/palea, two lodicules, six stamens, and a pistil extending from the periphery to the center (Fig. 4, C1–C4). With the exception of the glumes, numbers of four spikelet organ types were altered in the *Ubi1*:*AtJMT* spikelets (Fig. 4, D1–D7). For example, the numbers of lemma/palea and lodicules were increased, and these extra organs were often elongated. The number of stamens varied from five to 10 stamens. The number of pistils was increased, and a compound ovary with three stigma branches was observed. To investigate the developmental changes of *Ubi1*:*AtJMT* spikelets in detail,

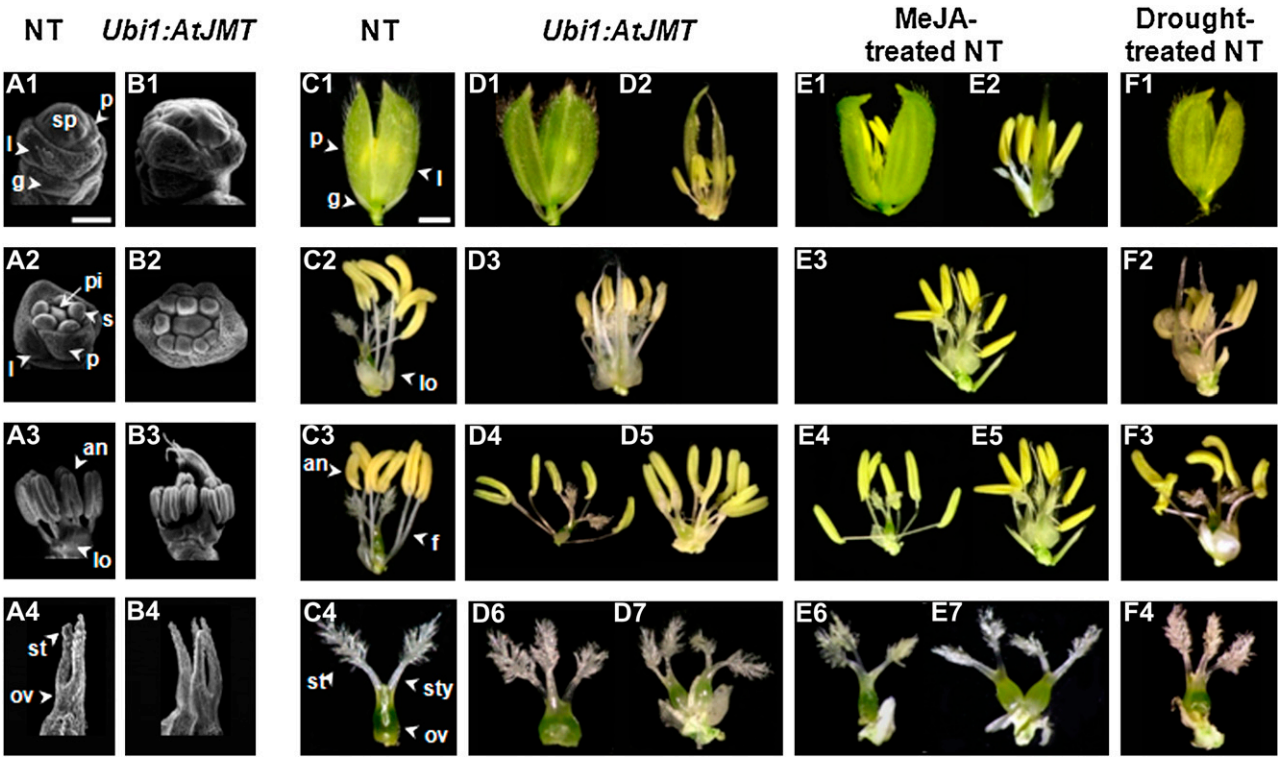

Figure 4. Spikelet morphology of NT, *Ubi1*:*AtJMT*, MeJA-treated NT, and drought-treated NT plants. Scanning electron micrographs of developing NT (A1–A4) and *Ubi1*:*AtJMT* (B1–B4) spikelets, a larger spikelet primordium (B1) than NT (A1), a spikelet with three extra stamen primordia (B2), a spikelet with extra stamens and extended lodicules (B3), and a spikelet with four stigma branches (B4). Light microscope images of NT (C1–C4), *Ubi1*:*AtJMT* (D1–D7), MeJA-treated NT (E1–E7), and drought-treated NT (F1–F4) spikelets. *Ubi1*:*AtJMT* spikelets with an extra whorl 1(D1), with lemma and palea removed showing two extra whorl 1 (D2), with normal lodicules and three extra, elongated lodicules (D3), with a stamen attached with two anthers (D4), with two extra stamens (D5), with a pistil with an extra stigma branch (D6), and with a gynoecium with an extra pistil (D7). MeJA-treated NT spikelets with an extra whorl 1 (E1), with two extra whorl 1 (E2), with extended lodicules (E3), with a stamen attached with two anthers (E4), with an extra stamen (E5), with a pistil with an extra stigma branch (E6), and with a gynoecium with an extra pistil (E7). Drought-treated NT spikelets with an extra whorl 1 (F1), with two elongated extra lodicules (F2), with an androecium with five stamens (F3), and with a pistil with an extra stigma branch (F4). an, Anther; f, filament; g, glume; l, lemma; lo, lodicule; ov, ovary; p, palea; pi, pistil; s, stamen; sp, spikelet primordium; st, stigma; sty, style. Bars = 50 μm (A1) and 1 mm (C1).

spikelets at the early stage of development were fixed and examined with a scanning electron microscope. Compared with the NT controls, the spikelet meristem of *Ubi1*:*AtJMT* plants was enlarged, the number of spikelet organ primordia was altered, and the extra organ structures were modified in appearance (Fig. 4, B1–B4).

The proportion of altered spikelets in *Ubi1*:*AtJMT* plants ranged from 34.8% to 60% (Table III). In NT plants that were treated either with exogenous MeJA or with drought, the proportion of altered spikelets was 11.1% or 10.0%, respectively. These proportions of altered NT spikelets were lower than those observed for *Ubi1*:*AtJMT* spikelets, possibly because MeJA levels were maintained at a high level throughout all stages of panicle development in *Ubi1*:*AtJMT* plants due to the constitutive expression of *AtJMT*, as opposed to transient increases of MeJA in NT plants. Thus, our results demonstrate that increased levels of MeJA in *Ubi1*:*AtJMT* plants are responsible for alteration in spikelet organ numbers.

Identification of Genes Regulated by MeJA and Drought in Young Panicles

To identify genes that are regulated by MeJA and drought, global expression profiling was performed on panicles from *Ubi1*:*AtJMT*, drought-treated NT, and untreated NT plants. The underlying assumption of

Table III. *Alteration in organ numbers of NT, Ubi1:AtJMT, and MeJA-treated and drought-treated NT spikelets*

Plants	Total[a]	Altered[b]	Percentage of Altered/Total
NT	640	3	0.5
Ubi1:*AtJMT*			
1-3-2	648	351	54.2
6-7-1	590	354	60.0
8-4-4	690	240	34.8
MeJA-treated NT	596	66	11.1
Drought-treated NT	856	86	10.0

[a]Number of total spikelets used in each assay. [b]Number of altered spikelets.

Table IV. *List of genes that are up- or down-regulated in Ubi1:AtJMT and drought-treated NT panicles as compared with untreated NT panicles*

Numbers appearing in boldface are those that are up- or down-regulated by more than 1.5-fold or more in *Ubi1:AtJMT* or drought-treated NT plants.

Gene Name	Accession No.[a]	*Ubi1:AtJMT*[b]	Drought-Treated NT[b]
Flavin-containing monooxygenase	AK071801	**3.2**	**3.4**
Ser/Thr kinase	AK069697	**7.3**	**3.5**
Hypothetical protein	AK059506	**5.1**	**4.3**
Ser carboxypeptidase	NM_001069939	**2.0**	**1.9**
Flavonol reductase	AK106089	**4.8**	**2.3**
Cyclin-like F-box domain-containing protein	AK068342	**2.2**	**3.7**
Hypothetical protein	AK063829	**4.5**	**2.8**
Flagelliform silk protein	AK062794	**5.0**	**1.5**
Floral nectary-specific protein (OsJMT1)	AK067321	**1.9**	**1.8**
OsSDR	AK064532	**1.9**	**2.4**
RING domain-containing protein	AK067013	**1.8**	**3.5**
Disease resistance protein	AK121159	**3.3**	−0.9
Galactose oxidase	AK060256	**6.8**	−0.7
Iron/ascorbate-dependent oxidoreductase	NM_001063498	**2.2**	−0.7
F-box protein interaction domain-containing protein	AK100978	**12.7**	−0.3
TPR-like domain-containing protein	AK069320	**1.7**	−0.1
Iron/ascorbate-dependent oxidoreductase	NM_001063495	**2.4**	0.4
Ser/Thr protein kinase	AK073168	**5.1**	0.6
MYB transcription factor (MYBS2)	AK071611	**2.0**	0.7
Leu-rich repeat-containing protein	NM_001052513	**2.4**	0.8
Pectinesterase family protein	AK106909	**5.8**	1.2
Cyclin-like F-box domain-containing protein	AK101804	**3.0**	1.4
Oxophytodienoic acid reductase (OPR)	AK072596	**2.6**	−1.0
Bowman-Birk proteinase inhibitor (BBPI)	AK102138	**4.8**	0.4
Disease resistance protein	NM_001060449	**2.3**	**−10.3**
Chitinase (OsChib3H-a)	AB027417	**4.4**	**−1.6**
WRKY61	NM_001075006	**2.0**	**−1.8**
Von Willebrand factor type A	AK070289	**2.1**	**−6.5**
Allyl alcohol dehydrogenase	AK060453	**−2.4**	**−2.3**
Met adenosyltransferase 1	NM_001049329	**−2.4**	**−2.1**
Acetyl-CoA synthetase	NM_001059240	**−2.0**	**−2.1**
RbcS	NM_001073137	**−1.8**	**−3.3**
WRKY45	AK066255	**−2.6**	**−2.2**
Cereal-type α-amylase inhibitor (α-amyl)	AK062381	**−2.0**	**−6.0**
PSII protein (PsbW)	AF366557	**−2.1**	**−2.6**
Isopenicillin nitrogen synthase	NM_001068348	**−1.6**	**−2.0**
Ser carboxypeptidase (SCP)	AK064814	**−2.4**	**−2.3**
RbcS	AK099574	**−2.3**	**−1.8**
Wound-induced protein	NM_001060540	**−2.1**	**−2.0**
Hsp70	X75616	**−3.3**	**−1.7**
Chlorophyll *a*/*b*-binding protein	AK062725	**−2.3**	**−1.5**
Glycoside hydrolase	AK061093	**−2.5**	−1.4
Small heat stress protein	AK119261	**−5.5**	−1.2
Naringenin-chalcone synthase family protein	AK119922	**−2.0**	−0.8
RbcS	AK068266	**−2.3**	−0.8
Chloroplast precursor (Chl)	AK120809	**−4.3**	−0.5
bZIP transcription factor (CAT103)	AK059435	**−1.9**	−0.5
Ethylene-responsive transcriptional coactivator	AK119729	**−2.7**	0.5
Terpene synthase family protein	AK067451	**−9.0**	0.5
Cyclin-like F-box domain-containing protein	NM_001074567	**−5.4**	**1.4**

[a]GenBank accession numbers for full-length cDNA sequences of corresponding genes. [b]The microarray data sets can be found at http://www.ncbi.nlm.nih.gov/geo/ (Gene Expression Omnibus). The Gene Expression Omnibus accession number of microarray data sets is GSE14508. Numbers represent means of relative expression levels of three independent biological replicates.

this approach was that high levels of MeJA produced either by overexpression of *AtJMT* in the transgenic panicles or by drought treatment in the NT panicles regulate genes that are involved in spikelet and/or panicle development. Profiling was conducted using the Rice 3′-Tiling Microarray (GreenGene Biotech). RNA samples from S1 panicles of *Ubi1*:*AtJMT*, drought-treated NT, and untreated NT plants were used to generate cyanine-3 (Cy3)-labeled cDNA probes, which were then hybridized to the microarray. Each data set was obtained from three biological repeats. When three replicates were averaged and compared with untreated NT panicles, 157 and 372 genes were up-regulated and 127 and 700 genes were down-regulated in *Ubi1*:*JMT* and drought-treated NT panicles, respectively.

From this list, we further selected genes that were regulated in both *Ubi1*:*JMT* and drought-treated NT panicles in all three replicates. The resulting collection of 10 up-regulated and 17 down-regulated genes is presented in Table IV. The regulated patterns of gene expression were subsequently confirmed by quantitative real-time PCR using the same RNAs that were used for the microarrays (Fig. 5). Some of the up-regulated genes have been reported to be associated with JA (Cheong and Choi, 2003) and ABA (González-Guzmán et al., 2002) biosynthesis, including 12-oxophytodienoate reductase (*OPR*), *OsJMT1*, and short-chain alcohol dehydrogenase (*OsSDR*). ABA levels are dramatically reduced in the *aba2* mutant of Arabidopsis, in which an ortholog of *OsSDR* has been knocked out (González-Guzmán et al., 2002), suggesting that OsSDR is a key enzyme for ABA biosynthesis. Senescence-related genes were also up-regulated in *Ubi1*:*AtJMT* panicles as compared with untreated NT panicles. These include *ERD1*, which mediates chloroplast protein degradation upon senescence in plants (Zheng et al., 2006; Jung et al., 2007), and the blue copper-binding protein gene that is activated during ozone exposure in Arabidopsis (Miller et al., 1999).

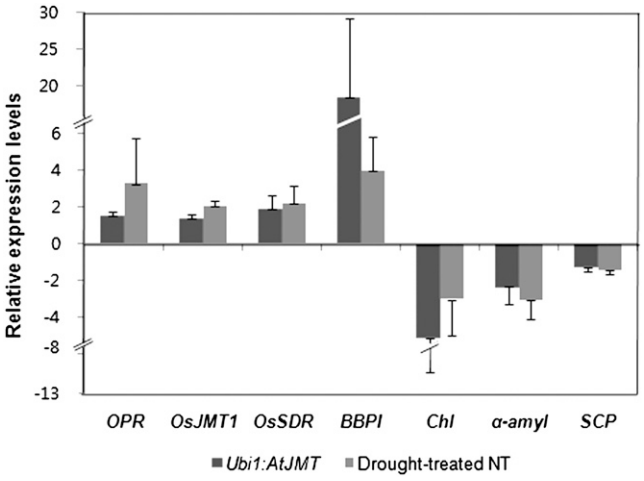

Figure 5. Relative transcript levels of regulated genes in *Ubi1*:*AtJMT* and drought-treated NT panicles relative to levels in untreated NT controls. Transcript levels of the regulated genes *OPR* (AK072596), *OsJMT1* (AK067321), *OsSDR* (AK064532), *BBPI* (AK102138), *Chl* (AK120809), *α-amyl* (AK062381), and *SCP* (AK064814) in the S1 panicles of *Ubi1*:*AtJMT*, drought-treated NT, and untreated NT plants were measured by quantitative real-time PCR. For each experiment, results were normalized using rice *tubulin* (AK102560) transcript levels. Values are means ± SD of three independent experiments.

DISCUSSION

In this study, we show that transgenic overexpression of the *AtJMT* gene in rice (*Ubi1*:*AtJMT*) increases MeJA levels in young panicles. Surprisingly, the increased MeJA levels caused dramatic changes in *Ubi1*:*AtJMT* plant reproductive development. These changes included a reduced number of spikelets per panicle, low filling rate, and alterations in floral organ numbers, collectively resulting in a large loss of grain yield. Similar phenotypes were also observed in NT plants that were treated with either exogenous MeJA or with drought at the panicle initiation stage, suggesting that MeJA is the key component for the observed alterations in reproductive development.

The effects of MeJA on plant development have also been examined in other model plant species. Unlike our *Ubi1*:*AtJMT* rice, Arabidopsis plants transformed with *35S*:*AtJMT* had increased levels of MeJA, but their flowers were visually indistinguishable from those of NT plants (Seo et al., 2001). This is likely because the anatomy and development of rice spikelets differ from those of Arabidopsis flowers (Ikeda et al., 2004; Dreni et al., 2007). Interestingly, Cipollini (2007) reported that Arabidopsis plants transformed with *35S*:*AtJMT* produced 40% less total seed weight than NT controls. In this case, the reduction in total seed weight was due to a reduced number of flowers. Similarly, application of exogenous MeJA on *Pharbitis nil* (Maciejewska and Kopcewicz, 2002) and *Nicotiana sylvestris* (Baldwin and Hamilton, 2000) led to a dramatic reduction in the number of flowers. Collectively, these reports from different plant species support our observations that the increased levels of MeJA in both *Ubi1*:*AtJMT* and drought-treated NT plants are responsible for the reduced number of spikelets per panicle.

Rice plants containing high levels of MeJA in their young panicles had not only a reduced number of spikelets but also a reduced filling rate. For example, in drought-treated NT plants, total seed weight was reduced by 48.8%. This reduction was the result of a 25% decrease in the number of spikelets per panicle and a 38.5% decrease in the filling rate as compared with results from untreated NT plants (Table II). The reduction in filling rate may have been due to altered levels of JA rather than MeJA. This possibility is supported by reports of decreased levels of JA, impaired floral development such as flower opening, and

impaired development and release of pollen in the Arabidopsis mutants *fad3-1/fad7-2/fad8*, *dde1*, and *dad1* (McConn and Browse, 1996; Sanders et al., 2000; Ishiguro et al., 2001). Lack of JA sensitivity in the Arabidopsis *coi1* mutant also led to male sterility (Xie et al., 1998). This does not appear to be the case in our *Ubi1*:*AtJMT* rice, in which levels of JA in young panicles remained unchanged. Similarly, JA levels were not altered in transgenic Arabidopsis and soybean overexpressing *AtJMT* and *NTR1* (a Chinese cabbage ortholog of *AtJMT*), respectively (Seo et al., 2001; Xue and Zhang, 2007). Thus, our results demonstrated that increased levels of MeJA altered the number of spikelet organs in *Ubi1*:*AtJMT* as well as in drought-treated NT plants. The alteration in spikelet organ numbers was correlated with a reduction in the filling rate in these plants, which subsequently led to a loss of grain yield. This possibility is supported by the reported observation that exogenous application of MeJA during the anther dehiscence stage of rice development resulted in increased sterility (Zhu et al., 2004). It is possible that the reductions in seed production of *Ubi1*:*AtJMT* plants were due partly to energetic costs associated with constitutive overexpression of *AtJMT*, overproduction of MeJA, and activation of defense and fitness responses to nutrients, as observed previously in Arabidopsis (Cipollini, 2007, 2009). Since MeJA stimulates the production of ABA in *Ubi1*:*AtJMT* plants, the reduction in grain yield was also due partly to ABA-mediated pollen abortion that was reported to occur under drought and cold stresses in rice and maize (Morgan, 1980; Oliver et al., 2007).

Application of exogenous MeJA was reported to inhibit *P. nil* shoot growth in a dose-dependent manner (Maciejewska and Kopcewicz, 2002). MeJA is also reported to inhibit seed germination, callus growth, and primary root growth (Ueda and Kato, 1982; Staswick et al., 1992; Berger et al., 1996; Nojavan-Asghari and Ishizawa, 1998). More recently, Pauwels et al. (2008) reported that high levels of MeJA repressed cell cycle progression by arresting cells in the G2 phase. It is likely that increased levels of MeJA reduce the number of spikelets in our *Ubi1*:*AtJMT* panicles by repressing cell cycle progression.

ABA is generally thought to play a role in plant response to drought stress. ABA was reported to increase by 1.8-fold in rice panicles in response to drought treatment at the mature panicle stage (Yang et al., 2001, 2004, 2007). In this study, we exposed NT plants to drought conditions for 2 weeks during the panicle initiation stage. In young panicles from drought-treated NT plants, the fold increase in levels of MeJA (19-fold) was much higher than that of ABA (1.4-fold) as compared with levels in untreated NT controls. In addition, in young panicles of *Ubi1*:*AtJMT* plants grown in nondrought conditions, levels of ABA were 1.9-fold higher than those of untreated NT controls, suggesting that ABA levels were increased due to MeJA exposure rather than by drought stress. These results led us to postulate that plants start to produce MeJA upon exposure to drought stress, which in turn stimulates the production of ABA. This hypothesis is supported by the fact that MeJA treatment increased ABA concentrations at an early stage of fruit development in sweet cherry (*Prunus avium*; Kondo et al., 2002). The *OsSDR* gene, an Arabidopsis ortholog of which is essential for ABA biosynthesis (González-Guzmán et al., 2002), was up-regulated in both *Ubi1*:*AtJMT* panicles and drought-treated NT panicles, suggesting its involvement in MeJA-dependent increases in ABA levels. The activity of the Arabidopsis *SDR* gene was reported to be required for JA biosynthesis (Adie et al., 2007), suggesting that ABA either precedes or cooperates with JA in signaling pathways. It is possible, therefore, that a positive feedback exists between MeJA and ABA under drought conditions. Determination of MeJA and ABA levels after exogenous application of ABA and MeJA or in ABA- and JA-defective mutants will clarify the feedback relationship further. Expression of *OsJMT1*, a rice ortholog of *AtJMT*, was increased in *Ubi1*:*AtJMT* and drought-treated NT panicles, indicating that *OsJMT1* may be responsible for the biosynthesis of MeJA upon exposure to drought conditions. *OsJMT1* shares 36% homology with *AtJMT* in nucleotide sequence without any stretch of 21 to 24 nucleotides that is required for a small RNA to potentially cross-react.

Overall, our results suggest that MeJA plays a role in a stress-induced loss of grain yield in rice.

MATERIALS AND METHODS

Plasmid Construction and Transformation of Rice

The expression plasmid *Ubi1*:*AtJMT* contained the *bar* gene under the control of the cauliflower mosaic virus *35S* promoter for use with herbicide-based plant selection. The *ubiquitin1* promoter, together with its intron (*Ubi1*), was used to drive constitutive plasmid gene expression (Christensen and Quail, 1996). The *AtJMT* cDNA clone was kindly provided by Dr. Y.D. Choi (Seo et al., 2001). Vectors were introduced into *Agrobacterium tumefaciens* LBA4404 by triparental mating. Embryonic callus initiated from the embryo of dehulled rice (*Oryza sativa* 'Nakdong') grains was transformed by cocultivation (Hiei et al., 1994), selected with 7 mg L^{-1} phosphinothricin, and used to regenerate transgenic plants according to the method of Jang et al. (2002).

Drought and MeJA Treatments

Transgenic and NT rice seeds were germinated in Murashige and Skoog solid medium in a growth chamber in the dark at 28°C for 3 d, transplanted to soil pots, and grown in a greenhouse (16-h-light/8-h-dark cycle) at 28°C to 30°C. Each pot (10 × 10 × 10 cm) was filled with rice nursery soil (Bio-media) according to the method of Oh et al. (2007, 2008). Twelve weeks after transplanting, rice plants (before panicle initiation) were subjected to either 2 weeks of drought or 60 μM MeJA (95%; Sigma-Aldrich) solution. When the plants had reached maturity and grains had ripened, the plants were harvested and threshed (separation of seeds from the vegetative parts). The unfilled and filled grains were taken apart, independently counted, and weighed. The following agronomic traits were scored: number of panicles, spikelets per panicle, filling rate (%), total seed weight (g), and 1,000 seed weight (g). Resultant data were separately analyzed by Student's *t* test for Table II. A spider plot of *Ubi1*:*AtJMT* and drought-treated NT plant agronomic traits as compared with untreated NT plants was drawn using Microsoft Excel software (Fig. 3).

Measurements of MeJA and ABA

Levels of MeJA were measured following the method of Engelberth et al. (2003). Approximately 0.5 g of S1 panicles was collected and extracted in methanol with an internal standard, [9,10-$^2H_2$]JA. Each sample was separated using a solid-phase extraction cartridge (reverse-phase C18, 12 mL; Mallinckrodt Baker), and sample pH was adjusted to pH 3.5 with 10% (v/v) formic acid. The oxylipin fraction was eluted with diethyl ether, and the eluate was completely dried under N_2 gas. Methanolysis was performed by incubation with a 1:2 (v/v) HCl:methanol mixture for 12 h at 30°C. The HCl:methanol was then completely removed under a stream of N_2 gas, and each sample was eluted with dichloromethane. Samples were analyzed by gas chromatography-mass spectrometry (Agilent Technologies).

Levels of ABA were measured following the method of Kang et al. (2005). Approximately 0.1 g of S1 panicles was collected and extracted with an extraction solution containing 95% isopropanol, 5% glacial acetic acid, and an internal standard, [(±)-3,5,5,7,7,7-d_6]ABA. The aqueous phase was extracted with ethyl acetate and separated using a silica cartridge (Sep-Pak; Waters Associates). The extracts were dried and methylated by adding diazomethane prior to gas chromatography-mass spectrometry (Agilent Technologies) analysis.

Scanning Electron Micrographs of Spikelets

Samples of panicles (<1 cm long) were prepared for scanning electron micrography analysis by prefixation in 0.1 M phosphate buffer (pH 7.4) containing 2.5% (v/v) glutaraldehyde and 4% (v/v) paraformaldehyde. Air was removed from the samples, and they were rinsed with phosphate buffer. Postfixation was carried out using OsO_4, and samples were dehydrated with 60% to 100% ethanol. The samples were treated with isoamyl acetate, dried, and ion coated. The mounted specimens were observed using an S-4300 scanning electron microscope (Hitachi).

Rice 3′-Tiling Microarray Analysis

Expression profiling was conducted using a Rice 3′-Tiling Microarray. Information on the microarray can be found at http://www.ggbio.com (GreenGene Biotech). The Rice 3′-Tiling Microarray was designed from 27,448 genes deposited at the International Rice Genome Sequencing Project RAP1 database (http://rapdb.lab.nig.ac.jp). Among these, 20,507 genes were from representative RAP1 sequences with cDNA/EST supports and 6,941 genes were predicted without cDNA/EST supports. Ten 60-nucleotide-long probes were designed from each gene, starting 60 bp ahead of the end of the stop codon with 10-bp shifts in position, so that 10 probes covered 150 bp in the 3′ region of the gene. In total, 270,000 probes were designed (average size, 60 nucleotides) to have Tm values of 75°C to 85°C. The microarray was manufactured by NimbleGen (http://www.nimblegen.com/). Random gas chromatography probes (38,000) were used to monitor the hybridization efficiency, and fiducial markers at the four corners (225) were included to assist with overlaying the grid on the image.

The microarray was used to profile gene expression in *Ubi1*:*AtJMT*, drought-treated NT, and untreated NT plants. Cy3-labeled target cDNA fragments were synthesized from S1 panicles using a Cy3-9mer primer. For normalization, data were processed with cubic alpine normalization using quartiles to adjust signal variation between chips and with Robust Multi-Chip Analysis using a median polish algorithm implemented in NimbleScan (Workman et al., 2002; Irizarry et al., 2003). To assess the reproducibility of the microarray analysis, we repeated the experiment three times with independently prepared total RNAs.

Real-Time PCR

Total RNA was prepared as reported previously (Oh et al., 2007). PCR products were amplified using the primers designed with Primer Designer 4 software (Sci-ed Software) listed in Supplemental Table S1. For quantitative real-time PCR experiments, the SuperScript III Platinum One-Step Quantitative RT-PCR system (Invitrogen) was used. For PCR, a master mix of reaction components was prepared according to the manufacturer's protocol for Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen). Thermocycling and fluorescence detection were performed using a Stratagene Mx3000p Real-Time PCR machine (Stratagene). PCR was performed at 95°C for 10 min, followed by 20 to 25 cycles of 94°C for 30 s, 57°C for 30 s, and 68°C for 1 min. To validate our quantitative PCR results, we repeated each experiment three times.

Supplemental Data

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

Supplemental Table S1. Primers used for real-time PCR.

ACKNOWLEDGMENT

We are grateful to Dr. Sang-Ik Song at Myongji University for making critical comments on the experimental results.

Received December 18, 2008; accepted February 4, 2009; published February 11, 2009.

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