Drought Stress Reduces Grain Yield by Altering Floral Meristem Development and Sink Size under Dry-Seeded Rice Cultivation

Cherryl Quinones, Nico Mattes, James Faronilo, Sudhir-Yadav,* and Krishna S.V. Jagadish*

ABSTRACT
Water and labor shortages are currently driving the shift from continuously flooded, puddled transplanted rice (*Oryza sativa* L.) (PTR-cf) to alternative crop establishment practices, such as dry-seeded rice (DSR). To improve water productivity by using DSR, fields are often kept under aerobic conditions. This shift could induce sensitivity to critical developmental processes during the early reproductive stages such as floral meristem (panicle) initiation and growth, when this coincides with water-limited conditions. To study the physiological impact of different establishment methods with different water available conditions, rice cultivar NSICRc 222 was evaluated, under PTR-cf, dry-seeded rice (DSR) with daily irrigation (DSR-d) and DSR with cyclic irrigation whenever soil water tension reached 10 kPa (DSR-10) and 40 kPa (DSR-40). Young developing inflorescences showed a morphological shift towards a slender shape under DSR-10 and a further increase in slenderness under DSR-40. We document a significant negative impact on developing inflorescence differentiation, a reduction in panicle neck diameter, and lower panicle nonstructural carbohydrate (NSC) content and grain length with a shift from PTR-cf to DSR-d. Key physiological traits such as retaining a globular-shaped floral meristem as a marker for optimal spikelet number and increased panicle sink size and panicle neck diameter, and NSC content for minimizing yield penalty are recommended when developing rice varieties for DSR, particularly under water-limited DSR conditions.

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Abbreviations: DAS, d after sowing; DSR, dry-seeded rice; DSR-10, DSR with cyclic irrigation to maintain 10 kPa soil moisture tension; DSR-40, DSR with cyclic irrigation to maintain 40 kPa soil moisture tension; NSC, nonstructural carbohydrate; PTR-cf, continuously flooded, puddled transplanted rice.

Across most rice-growing regions of Asia, irrigated rice is established by manual transplanting of seedlings into puddled soil (Pandey and Velasco, 1999), followed by prolonged flooding until shortly before harvest (PTR-cf). This method requires up to 2500 L of water to produce 1 kg of rice (Kreye et al., 2009; Mekonnen and Hoekstra, 2011) and a major proportion of the water is used during soil preparation for puddling. Water consumed by flooded rice is twice as high compared with other cereals, such as wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) (Peng et al., 2006). Increasing scarcity of irrigation water is rapidly becoming a major limiting factor for flooded rice production (Farooq et al., 2009). About 2 million ha of Asia’s dry-season irrigated rice area is estimated to face serious water shortages by 2025 (Tuong and Bouman, 2003). In addition, manual transplanting of rice requires cheap and readily available labor, which is becoming increasingly expensive and in high demand during the short peak planting period. The demand for a sustainable, less labor-intensive water-saving cultivation technology has resulted in recent adoption of DSR cultivation across South and Southeast Asia (Kumar...
and Ladha, 2011). For rice cultivation, this presents a two-dimensional change. First, the establishment method has to change from a nursery followed by transplanting to direct seeding in the main field (under wet or dry conditions); the second change is from a water management perspective: a shift from continuously flooded to aerobic or periodically saturated conditions similar to alternative wetting and drying technology.

Rice grown under DSR conditions has the potential to achieve yields of up to 6 t ha⁻¹ in the Philippines (Bouman et al., 2005; Peng et al., 2006; Sudhir-Yadav et al., 2014) or even 10 t ha⁻¹ in Japan (Kato et al., 2009), similar to PTR-cf. This can result in 12 to 33% lower water requirements (Kumar and Ladha 2011), but is highly dependent on proper water management and favorable environmental conditions such as precipitation and timely irrigation. On the other hand, aerobic conditions under DSR can negatively affect yield through increased weed pressure (Kumar and Ladha, 2011; Mahajan and Chauhan, 2013), nematodes (Prot and Matias, 1995), or micronutrient deficiency (Sudhir-Yadav et al., 2011). In addition, growing DSR in the tropics under realistic field conditions presents the danger of an imminent drift from a safe threshold of water availability to water-deficit stress if timely irrigation is not provided.

Abiotic stresses like drought and concurrent heat during the reproductive phase of rice have been shown to negatively influence different processes, depending on the developmental stage of their occurrence (Thakur et al., 2010; Rang et al., 2011; Jagadish et al., 2014a, 2014b; Shi et al., 2015). Although water-deficit stress during the flowering stage is primarily responsible for a reduction in spikelet fertility (Jagadish et al., 2010a, 2010b; Rang et al., 2011), stress impact during the very early reproductive stage, which affects active panicle morphogenesis and growth, could equally influence yield loss by altering adult panicle characteristics. Early development of the rice inflorescence is determined during the panicle initiation stage, approximately 30 d before heading. During panicle initiation and early growth of the inflorescence, primary, secondary, and tertiary branches are formed, which subsequently differentiate into terminal spikelets (Wang and Li, 2011). The process of panicle differentiation is typically completed in about 2 wk and hence any subsequent changes in spikelet number are identified to be solely caused by spikelet abortion during the early booting stage (Kato et al., 2008). Studies in japonica rice cultivars have shown that water-deficit stress during preflowering (but not precisely coinciding with panicle initiation) mainly affects the number of secondary branches, as well as postdevelopmental branch and spikelet abortion (Kato et al., 2008; Kato and Katsura, 2010). In addition to the direct impact on panicle morphogenesis, later reproductive stages, particularly flowering, could be potentially exposed to combined drought and heat stress because of reduced transpiration-driven increase in canopy and spikelet tissue temperature (Wassmann et al., 2009; Jagadish et al., 2011a, 2011b). In rice, this can lead to a significant increase in spikelet sterility or a decrease in grain quality and, subsequently, yield and economic loss (Lyman et al., 2013).

Under the emerging circumstances of potential global temperature increase and where classical irrigated rice cultivation becomes challenging because of reduced water availability, upland rice systems can serve as a resource-saving alternative. However, till date, systematic analysis to determine key physiological aspects that would threaten sustaining rice production with a transition from PTR-cf to DSR with different irrigation scheduling has not been attempted. Using DSR-d, as a bridge to PTR-cf and two distinct water limited conditions (i.e., DSR-10 and DSR-40) the objectives of our studies were to (i) systematically analyze the floral inflorescence development with a shift from PTR-cf to DSR-d and DSR-10 and DSR-40 (drought stress) conditions and the impact on sink size and (ii) quantify the impact of a transition from PTR-cf to DSR-d, DSR-10, and DSR-40 on panicle neck diameter, panicle temperature, and NSC partitioning to panicles, affecting yield and yield components.

**MATERIALS AND METHODS**

**Experimental Design**

The study was conducted at the IRRI upland research farm at Los Baños, Philippines (14°08’N, 121°15’E; 23 m asl) during the dry seasons of 2012 and 2013. The experimental site was composed of clay loam soil, up to 30 cm depth. The experiment was laid out with three replicates. Each subplot size was 16 by 4 m. There were four irrigation treatments: (i) PTR-cf, (ii) DSR-d, and irrigation when soil water tension increased to (iii) 10 kPa (DSR-10) and (iv) 40 kPa (DSR-40) at about 15 cm soil depth, maintained by following a cyclic irrigation method (Kumar et al., 2014; Venuprasad et al., 2009; Dixit et al., 2014). Previous studies at IRRI under similar soil conditions had consistently recorded 200 kg N ha⁻¹ as the optimum amount for achieving optimal yields and was thus used in this study. The PTR-cf treatment was topped up to 5 cm standing water depth whenever water depth fell to 1 cm or below. The water infiltration rate of the field was ~3 cm h⁻¹; hence, stagnation of water in the daily irrigated DSR-d treatment lasted for around 2 h. The amount of irrigation water applied to DSR-10 and DSR-40 treatments was 5 cm standing water depth every time they reached the target soil water tension. The approach was similar across both the years and the total amount of water applied and total rainfall throughout the crop growth season is included in Table 1.

**Crop Management**

The site was cultivated with two disc plowings followed by two rounds of a tractor-mounted rotovator (Model 399 4WD, Massey Ferguson, Tractorco Company Inc., Negros Oriental, Philippines) for DSR treatments. For PTR-cf, the soil
was soaked with water for 3 d followed by puddling. Seeds of ‘NSICRc222’ (a popular cultivar in the Philippines) were sown on 7 Jan. 2012 and 7 Dec. 2012 (for the 2013 experiment) for all three DSR treatments at 40 kg seeds ha\(^{-1}\) with a row spacing of 20 cm. The plant density varied between 150 and 200 plants m\(^{-2}\). Simultaneously, the seedbed for the PTR-cf treatment was sown. Next, 15-d-old seedlings were transplanted on 22 Jan. 2012 and 21 Dec. 2012 (for the 2013 experiment) in 20 by 20 cm planting geometry (25 seedlings m\(^{-2}\)). All treatments received a basal fertilizer (30 kg P\(_2\)O\(_5\) ha\(^{-1}\) as single superphosphate, 30 kg K\(_2\)O ha\(^{-1}\) as muriate of potash, and 10 kg Zn ha\(^{-1}\) as zinc sulphate) broadcasted prior to the last cultivation in DSR treatments or before the last round of puddling in PTR-cf. In addition, 200 kg N was applied in the form of urea in four equal splits at 15, 31, 45, and 60 d after sowing (DAS). In DSR, weeds were controlled by applying pre-emergence herbicide [oxadiazon (3-[2,4-dichloro-5-isopropoxyphenyl]-5-(2-methyl-2-propanyl]-1,3,4-oxadiazol-2[3H]-one) at 0.5 kg a.i. ha\(^{-1}\)] at 1 DAS, as well as manual weeding at 15 and 35 DAS. In PTR-cf, no herbicide was applied to control weeds; however, spot weeding was performed at 35 DAS. Furadan 3G (Quezon Farmer Agricultural Supply, Alaminos, Laguna, Philippines) (carbofuran, 2,2-dimethyl-2,3-dihydro-1-benzofuran-7-yl methylcarbamate) was applied at 20 DAS as a preventive measure to control nematodes. Irrigation treatments commenced from 33 DAS (three- to four-leaf stage) under DSR and 2 d after transplanting under PTR-cf. Irrigation was applied to keep the soil between saturation and 10 kPa up to the three- to four-leaf stage to avoid water stress during early establishment in all three DSR treatments. The DSR-d plots were irrigated daily and a cyclic irrigation schedule was followed whenever the soil water tension reached 10 kPa and 40 kPa for DSR-10 and DSR-40, respectively. The soil water tension was measured every morning at 0800 h using three tensiometers (Model R, Irrometer Company Inc., Riverside, CA) per treatment, installed with the center of the ceramic cup reaching 15 cm soil depth. Replicate plots of same treatment were irrigated simultaneously by pumping groundwater, channeled through a piped irrigation system with a separate outlet to each plot. The volume of irrigation applied to each plot was measured with a turbine flow meter (E-jet, Goal Team Trading, Quezon City, Philippines) and the depth of application was expressed in millimeters (Table 1).

### Grain Yield, Yield Components, and Physical Grain Dimensions

The yield components, straw, and grain yield were documented following the standard procedures of Cassman et al. (1994). At physiological maturity, rice plants from 12 designated hills (in PTR-cf) or four 0.6-m row lengths (in DSR) were harvested at ground level for recording yield components. At full harvest maturity, plants from 6 m\(^2\) (around the 12 hills taken for yield components) were harvested for determining grain yield. Grain yield (adjusted to 14% moisture) and straw weight from 6 m\(^2\) area was used to calculate harvest index by dividing grain yield (3% moisture content) over total aboveground dry matter.

Grain width was measured at the thickest point at the center of the grain, and grain length from the beginning to the end of the grain with a digital caliper (Mitutoyo Digimatic Caliper, Mitutoyo, Aurora, IL) (used for measuring panicle neck diameter), from 30 grains per replicate.

### Plant Sampling and Observations

Crop development was monitored and days to panicle initiation, 50% flowering, and physiological maturity were recorded during both the experiments (Supplementary Table S1). In the 2013 experiment, main tillers 3 m away from the border of each plot were tagged before maximum tillering stage to differentiate the primary tiller. Panicle initiation was determined by a series of meticulous destructive samplings from randomly chosen main tillers. The sampling of main tillers started on the 54th day after sowing, coinciding with the panicle initiation stage; sampling continued on alternate days until 20 d after panicle initiation, totaling to 10 sampling time points. Panicles were destructively extracted from surrounding stem tissue and preserved in 25% acetic acid and 75% ethanol for estimating panicle growth rate and microscopic analysis of the panicle meristem.

### Panicle Meristem Sectioning

The first two or three panicle meristem developmental stages (samples collected at panicle initiation and 2 or 4 d after panicle initiation) were obtained from the preserved samples mentioned above. Paraffin embedding and sectioning were performed to separate primary and secondary panicle branch differentiation. A total of three meristems at each stage were processed by dehydrating the meristems through a graded ethanol series (with 50, 60, 70, 80, 95, and 100% for 30 min each), infiltration with a xylene-ethanol series (3:1, 1:1, 1:3 xylene/ethanol for 30 min; and twice in 100% of xylene for 1 h), followed by embedding in paraffin (Paraplast Plus; Sigma Chemical Co., St Louis, MO). Sections of 10-μm thickness were obtained serially using a microtome (RM2135, Leica, Singapore) and the sections were placed on Superfrost Plus microscope slides (Fisher Scientific, Hampton, NH) and incubated at 45°C for 48 h. Incubated sections were dewaxed (twice in 100% xylene for 1 h; 1:3, 1:1, 3:1 xylene/ethanol for 10 min each), rehydrated through a graded ethanol series (100, 95, 80, 70, 60, 50% for 1 min each) and later stained with 2% safranin dissolved in 50%
ethanol for 30 min. The sections were then washed in ethanol and subsequently stained with 0.05% Fast Green (dye content ≥85%) in 80% acetone for 15 s and washed with isopropanol and 100% xylene. The samples were then mounted with cover slips and oven-dried at 65°C for 24 h. Sections were viewed under an Axiosplan 2 microscope (Carl Zeiss, Oberkochen, Germany) and images taken using an Olympus DP70 camera (Olympus, Tokyo, Japan) attached to the microscope following Jagadish et al. (2014a). Taking the ratio between bract diameter (right below the first primary branch) and panicle meristem length from the base of the bract to the tip of the youngest panicle branch provided a coefficient of panicle shape during early development (Supplementary Fig. S1). The ratio between panicle meristem length over its base diameter was used to determine whether the meristem was globular or slender.

Panicle Growth Rate and Panicle Neck Diameter
In 2013, the temporal increase in the length of the developing panicle from neck node to the tip of the topmost spikelet was measured on alternative days before booting, from 8 d to 20 d after panicle initiation, using the samples mentioned in the Plant Sampling and Observation section. At least three replicate panicle samples were used for this measurement. Panicle neck diameter was measured at 1 to 2 cm below the neck node at physiological maturity from 30 main tiller panicles for each treatment (10 panicles per replication) using a Mitutoyo Digital Caliper (Mitutoyo Corporation, Kawasaki, Japan).

Canopy, Panicle, and Soil Temperature
Canopy temperature was taken in all plots with three replicates per subplot, using an IR Crop Temperature Meter (Spectrum Technologies, Inc. IL) in 2013. Measurements were taken on 1 Mar. 2013 with a mean day temperature of 26.8°C (maximum temperature, 30.6°C). The instrument was pointed at the rows of the plants from about 1.5 m to 2 m away at a uniform angle of 45° between 1000 and 1100 h. Three biological repeats of panicle temperature per subplot were taken between 1000 and 1200 h during the flowering stage using an infrared thermal camera (INFR.EC R.300 NEC AVIO, NEC AVIO Infrared Technologies Company, Japan) at 10 to 15 cm from the target panicle (Julia and Dingkuhn, 2012). The measurements were divided into upper, middle, and lower points along the panicle to ascertain the gradient in temperature difference at the top (surrounded by less leaf canopy) and lower (surrounded by thicker leaf canopy). Soil temperature was logged with a waterproof Temperature/Light Data Logger (Doc # 9556F, MAN-UA-002, Onset Computer Corporation, MA). One soil temperature logger per plot was buried at about 20 cm depth, and temperature was logged at 15-min intervals, starting from panicle initiation until maturity. All soil, canopy, and panicle temperatures were in °C.

Nonstructural Carbohydrate
Grains from the tagged main tillers, collected at maturity, were used to spectrophotometrically quantify the soluble sugars and starch content after ethanol extraction, following Yoshida et al. (1976).

Statistical Analysis
In 2012 and 2013, a randomized block design was followed and the treatments were imposed on the same plots in both the years. Data collected for all the parameters above were analyzed for significance of variation. Comparative regressions for quantifying the panicle growth rate were obtained using Genstat (14th edition, Rothamsted Experimental Station, Harpenden, UK).

RESULTS
Grain Yield and Yield Parameters
In both years, there was significant effect of irrigation scheduling on the grain yield of rice. The grain yield was highest with PTR-cf, which did not differ significantly from DSR-d but was significantly higher than DSR-40 in both years higher than DSR-10 only in 2013 (Table 2). There was a 11 and 17% yield decline with DSR-10 compared with DSR-d and PTR-cf, respectively in 2013. The yield penalty further increased to 19 to 28% and 34 to 38% with DSR-40 compared with DSR-d and PTR-cf during 2012 and 2013, respectively (Table 2). Higher yield in PTR-cf was influenced significantly by the higher spikelet number per panicle and the average grain weight, whereas the higher number of panicles contributed most significantly to yield under DSR-d.

The panicle density was significantly higher under DSR-10 and DSR-40 treatments than PTR-cf in 2012 but was statistically similar in 2013. Contrary to this, spikelets per panicle were significantly higher under PTR-cf than DSR. Spikelets per panicle were about 28% lower (average of 2 yr) under DSR-d than under PTR-cf and the number further increased to 48% with water stress (DSR-40). Percentage of seed set was similar in PTR-cf and all DSR treatments. The average grain weight was significantly affected by drought stress, with PTR-cf recording an average 1000-grain weight varying between 22 and 23 g, which was statistically similar to that of DSR-d. However, average 1000-grain weight was reduced by 9 and 11% for DSR-10 and DSR-40, respectively compared with PTR-cf. The average panicle length was reduced by 2 and 3% for DSR-10 and DSR-40, respectively compared with PTR-cf, which was statistically similar to that of DSR-d. However, the average grain weight, whereas the higher number of panicles contributed most significantly to yield under DSR-d.

Phenology, Canopy and Soil Temperature, and Soil Water Tension and Irrigation Input
All plots reached both panicle initiation and physiological maturity at similar time points in both years (Supplementary Table S1). Cyclic irrigation was provided in the DSR-10 and DSR-40 starting at 25 and 33 DAS in 2012 and 2013, respectively, with a focus on maintaining the targeted stress level. The nonstressed DSR-d and PTR-cf fields remained under saturated and fully flooded conditions, respectively.
Table 2. Grain yield, harvest index, yield components and grain physical dimensions of NSICRc222 rice under different irrigation scheduling during the dry season of 2012 and 2013.

<table>
<thead>
<tr>
<th>Year</th>
<th>Irrigation schedule</th>
<th>Grain yield (kg ha(^{-1}))</th>
<th>Harvest index</th>
<th>Panicle number (per m(^2))</th>
<th>Spikelet number (per panicle)</th>
<th>Seed-set (%)</th>
<th>1000-grain weight (g)</th>
<th>Grain length (mm)</th>
<th>Grain width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2012 DS</td>
<td>PTR-cf‡</td>
<td>6437 a</td>
<td>0.48 a</td>
<td>326 b</td>
<td>111 a</td>
<td>82</td>
<td>23 a</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>DSR-d</td>
<td>5668 ab</td>
<td>0.48 a</td>
<td>438 ab</td>
<td>97 a</td>
<td>85</td>
<td>22 a</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>DSR-10</td>
<td>5780 ab</td>
<td>0.44 ab</td>
<td>465 a</td>
<td>80 b</td>
<td>77</td>
<td>20 b</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>DSR-40</td>
<td>4605 b</td>
<td>0.39 b</td>
<td>506 a</td>
<td>76 b</td>
<td>66</td>
<td>20 b</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>5622</td>
<td></td>
<td>434</td>
<td>91</td>
<td>78</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F (prob)</td>
<td></td>
<td>0.047</td>
<td>0.031</td>
<td>0.041</td>
<td>0.006</td>
<td>0.136</td>
<td>0.001</td>
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</tr>
<tr>
<td>LSD (0.05)</td>
<td></td>
<td>1185</td>
<td>0.06</td>
<td>117</td>
<td>16</td>
<td>ns</td>
<td>1.15</td>
<td></td>
<td></td>
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<tr>
<td>2013 DS</td>
<td>PTR-cf†</td>
<td>7561 a</td>
<td>0.50 a</td>
<td>360</td>
<td>157 a</td>
<td>78</td>
<td>22 a</td>
<td>7.02 a</td>
<td>1.71</td>
</tr>
<tr>
<td></td>
<td>DSR-d</td>
<td>7070 a</td>
<td>0.45 b</td>
<td>440</td>
<td>95 b</td>
<td>72</td>
<td>22 a</td>
<td>6.9 a</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>DSR-10</td>
<td>6265 b</td>
<td>0.46 b</td>
<td>495</td>
<td>74 b</td>
<td>74</td>
<td>21 b</td>
<td>6.74 b</td>
<td>1.68</td>
</tr>
<tr>
<td></td>
<td>DSR-40</td>
<td>4662 c</td>
<td>0.40 c</td>
<td>472</td>
<td>62 b</td>
<td>75</td>
<td>20 c</td>
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<td>1.66</td>
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<tr>
<td>Mean</td>
<td></td>
<td>6390</td>
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<td>97</td>
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<tr>
<td>F (prob)</td>
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<td>&lt;0.001</td>
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<td>0.031</td>
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<td>0.013</td>
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</tr>
<tr>
<td>LSD (0.05)</td>
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<td>785</td>
<td>0.01</td>
<td>ns</td>
<td>59</td>
<td>ns</td>
<td>0.77</td>
<td>0.17</td>
<td>ns</td>
</tr>
</tbody>
</table>

† Means with the same letter are not significantly different at 5% probability according to LSD.
‡ PTR-cf, continuously flooded, puddled transplanted rice; DSR-d, dry-seeded rice with daily irrigation; DSR-10, dry-seeded rice irrigated at 10 kPa soil moisture tension; DSR-40, dry-seeded rice irrigated at 40 kPa soil moisture tension; ns, not significant.

More detailed documentation of the soil and canopy temperature was performed only in 2013. It took, on average, 3 to 4 d to reach 10 kPa and 4 to 5 d to reach the desired water tension level of 40 kPa, except for the first cycle, which took 7 d for 10 kPa and more than 10 d to reach 40 kPa, mainly because of 73 mm of rain received immediately after the onset of the stress treatment (Fig. 1A). The amount of irrigation applied was significantly higher for DSR-d than the rest of the treatments in both years (Table 1). The irrigation amount was similar in PTR-cf and DSR-10 but significantly lower in DSR 40 in 2012. In 2013, the irrigation amount for PTR-cf was significantly less than that for DSR–10 but on a par with that for DSR–40.

A 1 and 3°C increase in canopy temperature for DSR-10 and DSR-40, respectively was recorded as the treatments reached their target water tension levels, compared with DSR-d (Fig. 1B). PTR-cf and DSR-10 recorded similar canopy temperatures and the former was used as a reference to compare the other three treatments. Peak canopy temperature was recorded at 94 DAS during the eighth drying cycle, which coincided with the early grain-filling stage. The daytime soil temperature increase coincided with the peak water-limiting phase during the drying cycle and recorded about a 1 to 2°C increase with DSR-10; a 2 to 4°C increase was more consistent under the DSR-40 treatment (Fig. 1C). The daytime soil temperature of PTR-cf, on the other hand, remained 1 to 2°C cooler than that of DSR-d because of constant standing water and equal temperature between these two treatments during nighttime. Average nighttime soil temperature was only slightly elevated (by 0.5°C) in DSR-40 plots. The only other rain event occurred at 77 and 78 DAS (129 mm), which coincided with the start of the flowering stage.

### Panicle Growth Rate

A comparative regression during the panicle growth period, using DSR-d as a reference, resulted in similar growth rate as PTR-cf and DSR-10 (P > 0.05) but a significantly slower growth rate for DSR-40 (P < 0.05) (Fig. 2A). Panicles from the DSR-10 and DSR-40 treatments not only showed a delayed start of the maximal elongation phase but that this stage also plateaued with a 2-d delay. Although the DSR-40 panicles ultimately attained similar lengths to those in DSR-10, the rate of growth was much slower. Average panicle length on the 20th day after panicle initiation was lower by 2.1 cm (SE = ± 0.4 cm) in DSR-10 and 3 cm lower (SE = ± 0.6 cm) in DSR-40 than in DSR-d. Final panicle length (Fig. 2B) from the main tillers at maturity showed a significant treatment-dependent decrease (P < 0.05) from PTR-cf to DSR-d (4.6% reduction) and from DSR-d to DSR-10 and DSR-40 with 9 and 13% reductions, respectively.

### Panicle Meristem Morphology, Spikelet Number and Panicle Neck Diameter

Paraffin sections of very early panicle meristems (1–3 d after panicle initiation) showed clear differences in shape and morphology between treatments (Fig. 2 C,D). The PTR-cf panicles recorded a lower ratio of length to base (P < 0.05; 95% confidence interval ranging between 1.70 and 1.70), indicating a more compact and shorter meristem than DSR-d panicles (95% confidence interval ranging between 1.60 and 2.12). Drought treatment increased the ratio further, recording the longest and most slender panicles (95% confidence interval ranging between 1.8 and 2.4).

Primary rachis branch number was only slightly affected among the tested treatments (P < 0.05), whereas a greater (P < 0.001) decline in secondary branches was
observed between PTR-cf and DSR-d treatments (Fig. 3A). The PTR-cf treatment recorded nearly double the number of secondary branches compared with the DSR treatments on average. All DSR treatments recorded a greater reduction of secondary branches with a 29% decrease in DSR-10 and 42% in DSR-40 compared with DSR-d. Spikelet number differed significantly across the treatments \((P < 0.001)\), with DSR-d showing a 39% decrease compared with PTR-cf (Fig. 3B). Both DSR-10 and DSR-40 recorded a further decrease in spikelet number by 31 and 32%, respectively, compared with DSR-d.

Similarly, panicle neck diameter recorded a significant response to water treatments \((P < 0.001)\) (Fig. 3C). The DSR-d treatment recorded a 17% decrease in panicle neck diameter compared with PTR-cf. Further, the DSR treatments recorded 11% (DSR-10) and 19% (DSR-40) smaller neck diameter, respectively, compared with DSR-d. A regression analysis between main tiller panicle neck diameter and spikelet number shows a strong linear relationship between both the variables, with defined clustering for all treatments, except for a slight spread in DSR-d, which mainly ranged between the PTR-cf and DSR-10 clusters (Fig. 3D).

Fig. 1. Treatment details and plant response. Monitored soil moisture tension (A), canopy temperature (B), soil temperature (C), maximum ambient temperature (D), and their variation throughout the stress exposure period. Crop growth in d after sowing (DAS) is overlaid on top of each of the three panels to follow the phenological stages during the treatment period. Canopy and soil temperature changes under the continuously flooded, puddled transplanted rice (PTR-cf) and dry-seeded rice irrigated at 10 kPa soil moisture tension (DSR-10) and dry-seeded rice irrigated at 40 kPa soil moisture tension (DSR-40) treatments are relative to dry-seeded rice with daily irrigation (DSR-d) recorded between 53 and 72 DAS, and between 82–105 DAS for canopy temperature and from 54 to 90 DAS for soil temperature. The gap in the relative canopy temperature curve was caused by a technical breakdown in the temperature recording unit. The area between the x axis and the plot curve for soil temperature has been darkened for visibility.
Fig. 2. Panicle formation dynamics. Prebooting rice panicle elongation, mature panicle length, and pre-elongation panicle shape for main tillers compared under different growth conditions. Changes in panicle length during elongation time compared between water treatments (A), panicle length at maturity stage compared between treatments (B), evaluation of the shape of <1-mm panicles using the length/diameter quotient (C), and paraffin sections (10 µm) using <1-mm panicles, illustrating the change in meristem morphology between crop establishment methods and water availability levels (D). Scale bar = 200 µm. Error bars indicate ± SE. PTR-cf, continuously flooded, puddled transplanted rice; DSR-d, dry-seeded rice with daily irrigation; DSR-10, dry-seeded rice irrigated at 10 kPa soil moisture tension; DSR-40, dry-seeded rice irrigated at 40 kPa soil moisture tension; PI, panicle initiation.

Fig. 3. Changes in main panicle architecture traits in rice. Number of primary and secondary branches from panicles grown under different conditions (A), total spikelet number per panicle including filled and unfilled spikelets (B), panicle neck diameter (C), and relationship between spikelet number per panicle and neck diameter under different growth conditions (D). $y = 152.04x - 165.26$, $R^2 = 0.82$, $n = 30$. Bars indicate SE. PTR-cf, continuously flooded, puddled transplanted rice; DSR-d, dry-seeded rice with daily irrigation; DSR-10, dry-seeded rice irrigated at 10 kPa soil moisture tension; DSR-40, dry-seeded rice irrigated at 40 kPa soil moisture tension.
Panicle Tissue Temperature

An increasing gradient in temperature was observed from the base to the top of the panicle across all four treatments (Fig. 4). The bottom portion showed no significant fluctuation ($P > 0.05$), although the middle and the top portion recorded a significantly higher panicle tissue temperature ($P < 0.05$). Panicle tissue temperature at flowering did not differ between PTR-cf and DSR-d but increased significantly under DSR-10 ($+2 \pm 0.18^\circ C$, $P < 0.05$) and DSR-40 ($+2.4 \pm 0.08^\circ C$, $P < 0.01$). Measurements in the top one-third of the panicle differentiated panicle temperature across the treatments with 1 to 2°C higher values than in the lower one-third of the panicle. The temperature gradient along the panicle became more prominent under decreased water availability conditions, with temperatures reaching up to 37°C in the upper section (Fig. 4). Maximum air temperature and radiation for this peak day were 30.6°C and 22.8 MJ m$^{-2}$, respectively. Panicle tip portions even recorded peak temperatures of 39°C under DSR-40 conditions.

Panicle NSC

Sugar and starch content distribution in main tiller panicles at maturity showed differential distribution between DSR treatments (Fig. 5). Both DSR-10 and DSR-40 accumulated significantly lower ($P < 0.05$) panicle sugar content. Similarly, the starch content was also lowered ($P < 0.01$) by 20 and 31% under DSR-10 and DSR-40, respectively, relative to DSR-d. No difference was observed between PTR-cf and DSR-d for panicle sugar or starch content.

DISCUSSION

Similar grain yield can be obtained under DSR-10 and PTR-cf in the eastern plains of India with sufficient rainfall, whereas in the northwest, yield penalty is primarily a result of low precipitation [1000–1500 mm (east) vs. 400–750 (northwest)] (Singh, 2008). Given the predicted warmer and drying climate with fewer rainy days (Intergovernmental Panel on Climate Change, 2013), the probability of exposure to intermittent drought stress is an additional challenge for the effective adoption of DSR-10 in tropical rice-growing regions. Previous studies have identified improved crop establishment, better weed competitiveness, higher spikelet fertility, and lodging resistance as key traits adapting rice to DSR-10 (Khush, 1995; Ahmed et al., 2014). We identified sensitivity of the panicle during morphogenesis as well as during grain filling to be vulnerable with DSR under water-limited conditions. In our study, the comparison between PTR-cf and DSR is not straightforward, since they differ not only in water availability and aerobic soil status but also differ significantly in planting density. Hence, comparisons were made between PTR-cf and DSR-d to differentiate the impact of management system and between DSR-d and both DSR-10 and DSR-40 to quantify the impact of water-limited stress under aerobic conditions.

The transition from PTR-cf to DSR-d did not lead to an increase in soil, canopy, or panicle temperature (Fig. 1 and Fig. 4). However, the water required to provide such an environment for DSR-d was more than double (Table 2) that of conventional PTR-cf because of the lack of a hard pan and therefore, DSR-d did not meet the purpose of developing a water-saving technology (Sudhir-Yadav et al., 2011; Mandal et al., 2013). Hence, having 10 kPa in DSR-10 as a target environment is considered appropriate and practically feasible (Kreye et al., 2009, Bouman and Tuong, 2001). Shifting from DSR-d to DSR-10 increased soil, canopy, and panicle temperatures by about 2°C. This difference was pushed even further with the introduction of drought stress under DSR-40. The higher soil temperature recorded at 20 cm below the surface, indicates the possibility of an even higher temperature close to the soil surface, which is near the developing panicle during the early panicle initiation stage, which was not captured in our experimental setup.
Increasing the severity of drought stress has been shown to lead to increased spikelet sterility or seed-set reduction because of lower transpiration cooling, resulting in increased panicle tissue temperature (Garrity and O’Toole, 1994; Jagadish et al., 2011a; Shi et al., 2015). A similar phenomenon was not observed in our study, in spite of an increase in the panicle microclimate temperature, as shown by the lack of a reduction in seed set. This could be because of a 8-d break in the drying cycle resulting from 129 mm of precipitation between 77 and 78 DAS, which coincided with the critical flowering stage. In addition, the temperatures documented were not consistently higher than the defined critical threshold of ≥35°C to induce spikelet sterility (Jagadish et al., 2010a, 2010b). To test this hypothesis, evaluating the conditions imposed in this study under hotter and drier conditions where temperatures exceeds the critical threshold would help to capture the proportion of an increase in spikelet sterility caused by drought stress–driven increases in tissue temperature. The panicle neck is the checkpoint between source and sink and its diameter is shown to be associated with many agronomic traits, including panicle size. It is also associated genetically with panicle number, panicle length, primary branch number, secondary branch number, spikelet number per panicle, spikelet density, and grain number per panicle both under well-watered and drought stress conditions (Liu et al., 2008). Larger panicle neck diameter is also positively correlated with the xylem cross-section accommodating large vascular bundles, which facilitates conduction of a large volume of water (Xu et al., 2000). Under conditions with higher ambient temperatures, a higher transpiration cooling demand will be challenged by the narrow panicle neck diameter, thus essentially increasing panicle tissue temperature further, which could induce greater spikelet sterility.

Grain number is regarded as one of the major determining factors of crop yield and it is frequently targeted for crop improvement (Horie et al., 2005; Fujita et al., 2012). However, the dynamics of spikelet formation and subsequent grain maturation are highly dependent on environmental factors such as N supply (Wada, 1969) or water management (Kato et al., 2006). Most previous attempts to genetically increase grain number are reported under PTR-cf conditions (Fujita et al., 2012); this and other related traits are also starting to be evaluated under aerobic conditions. Adopting new cropping techniques such as DSR may influence the genetic response that determines spikelet number compared with fully irrigated conditions, which has not been systematically investigated to date.

This study highlights possible mechanistic factors that could determine rice grain yield losses even under fully saturated (DSR-d) and water-limited (DSR-10 and DSR-40) conditions. Though PTR-cf and DSR-d showed similar panicle elongation dynamics, the shape of <1-mm-sized developing panicle meristems showed a more compact developing panicle under PTR-cf and a more slender panicle under DSR-d with the slenderness increasing with increasing water limitation under DSR-10 and DSR-40. This change in early shape of the panicle meristem corresponds to architectural changes that were observed later in the adult panicle, with secondary branches reduced by almost half and spikelet number by one-third. These results indicate that environmental conditions influence the early stages of panicle development. In addition, a strong decline in panicle neck diameter was observed with the move towards DSR and this corresponded with a significantly lower panicle NSC content, a potential factor for a programmed response to reduced seed filling and grain weight, which was associated more with grain length reduction than grain width. The larger vascular tissue (Xu et al., 2000) associated with bigger panicle neck diameter has been shown to play a key role in assimilate transport to the grains (Cui et al., 2003). Hence, the reduced panicle neck diameter and the lower panicle NSC provides a mechanistic explanation for the lower grain length and the large reduction in grain weight, particularly under DSR-40. Future studies should consider determining the rate of grain filling, which will help to quantify the NSC translocation and the grain filling dynamics. Taken together, the results obtained in this study show that severe morphological differences occur between PTR-cf and DSR-d during the early panicle developmental phase, but through counteracting trade–off traits, the multifactorial yield is balanced out, but not under exposure to drought stress (DSR-40). Additionally, this compensation response between PTR-cf and DSR-d on yield could be a direct result of higher plant density (25 vs. 175 plants m⁻², respectively).

In summary, we demonstrate the chain of events that could potentially be affected by the transition from PTR-cf to DSR-10, such as altered panicle morphology leading to lower secondary rachis branches and, in turn, spikelets per panicle, reduced panicle neck diameter, lower panicle NSC, and reduced grain length, resulting in reduced yield. Progress achieved in drought-stress breeding programs (Venuprasad et al., 2009; Ye et al., 2012; Kumar et al., 2014) can be extended to DSR-10 and DSR-40 to overcome stress damage at the critical reproductive stage. In addition, emphasis on key recommended traits, such as retaining a globular panicle meristem, increased sink size, larger panicle neck diameter, and higher panicle NSC would allow continued adoption of DSR technology in current and future drier tropical rice-growing regions. The findings provide a starting point for further wider testing of this comparison involving multiple entries and soil types in tropical and subtropical rice growing regions. Mechanistically, not much is known about how the meristem cells, which generate the entire panicle architecture, respond to drought or combined drought and heat stress.
at the genetic and metabolic level, which is timely and an interesting area of research.

**Supplementary Materials**

Supplementary Figure S1. Measurements taken from young rice panicle meristem microsections included (a) panicle length from the base of the lowest primary branch to the panicle tip, (b) base diameter at the height of the lowest primary branch, (c) number of primary branches, and (d) the number of secondary branches in the section plane.

Supplementary Table S1. Sowing, transplanting and key phenological developmental time-points in relation to sowing under different cultivation conditions. PTR, puddled transplanted rice; DSR, direct seeded rice; CF, continuously flooded; PI, panicle initiation.

**Conflict of Interest Disclosure**

The authors declare no conflicts of interest.

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