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Genotypic consistency for low temperature tolerance at the booting stage in rice grown under flooded and non-flooded conditions

L.V. Ha*, J.H. Mitchell, S. Fukai

The University of Queensland, School of Agriculture and Food Sciences, St Lucia, Qld 4072, Australia

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ABSTRACT

Lack of irrigation water for flooded cultivation and low temperature during the reproductive phase are factors limiting rice production in the Riverina region of New South Wales, Australia. While cold tolerant genotypes are available for flooded conditions, the consistency of cold tolerance under different growing conditions including non-flooded conditions remains unknown. The objective of this paper was to determine genotypic consistency in cold tolerance under different water and soil nutrition conditions. Three experiments were conducted in which genotypes from a Kyeema//Kyeema/Norin PL8 population were exposed to low air temperature (21/15 °C day/night) for 14 days at the booting stage. Genotypic variation in spikelet sterility existed within the population and the ten most tolerant (22.8%) and susceptible (73.2%) genotypes were identified. Generally, the genotypes grown under non-flooded conditions and low temperature ranked similarly to those in the flooded and low temperature (r = 0.90**), with tolerant genotypes consistently identified as tolerant even under high nitrogen flooded (r = 0.97**) conditions. N application increased tillering and spikelet number per plant, leading to increased spikelet sterility under low temperature. Spikelet sterility negatively correlated with grain yield/plant but some genotypes with low spikelet sterility also had low grain yield, due to inherently low spikelet number per panicle. The genotypic consistency in cold tolerance under different growing conditions suggest that it may be possible for genotypes to be screened for low temperature tolerance under flooded conditions and identify genotypes appropriate for use in non-flooded conditions, thus minimising resources required to screen for low temperature tolerance and non-flooded conditions in a two-step process. However, to confirm this, testing in the field with larger population numbers from diverse genetic backgrounds is required.

1. Introduction

Rice (*Oryza sativa* L.) is widely grown as an annual crop in many countries in both temperate and tropical zones, from latitudes ranging from 53°N to 40°S (Lu and Chang, 1980). Low temperature is a serious problem and capable of reducing rice grain yields in many temperate zones and high altitude regions (Mackill and Lei, 1997). Throughout the world, low temperatures have been estimated to cause an annual grain loss of about 10% (Garg et al., 2003). Low temperature stress affects all growth stages of rice, from germination to grain maturity (Andaya and Mackill, 2003; Ye et al., 2009). The duration of the cold exposure is also an important factor that determines the effects of low temperature on rice yield. The most critical stage for low temperature effect is the young microspore stage, which occurs during booting (Gunawardena et al., 2003a; Farrell et al., 2006b; Shimono et al., 2007; Ye et al., 2010). During the booting stage, exposure to low temperature results in increased spikelet sterility and reduced grain yield (Heenan 1984; Satake 1976). The high spikelet sterility is often associated with the failure of the developing pollen grains when low temperatures occur in the microspore development stage (Heenan, 1984; Gunawardena et al., 2003b; Farrell et al., 2006b; Shimono et al., 2007).

Spikelet sterility determined at maturity is the most common trait used to identify low temperature tolerant genotypes in rice (Satake and Shibata, 1992). Satake and Shibata (1992) exposed 19 japonica varieties to a constant day/night air temperature treatment of 12 °C for 3 days at the booting stage and found that spikelet sterility varied from 17 to 79%. Similarly, Farrell et al. (2006a) exposed 23 rice varieties to cool water (maintained at about 19 °C for 25 days) at the booting stage and the resulting spikelet sterility ranged from 8% to 70% among varieties. However, most studies on genotypic variation to date, have used varieties with widely different genetic backgrounds, and the use of genotypes from a population is limited. Although, one study recently reported by Mitchell et al. (2016) who was working on 20 Reiziq × Lijiangheigu genotypes from extreme phenotypic bulks, identified tolerant and susceptible genotypes ranging from 12 to 100% sterility when exposed to 24/15 °C day/night temperatures for 14 days

* Corresponding author.
E-mail address: vietlongtuaf@yahoo.com.vn (L.V. Ha).

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at the booting stage.

In Australia, where commercial rice has been grown traditionally under flooded conditions in Southern New South Wales Riverina region, one management method currently being utilized to minimize low temperature exposure at the early microspore stage is the use of flooding with a 25 cm 'water blanket', which provides a temperature buffer to protect the developing floral structures (Farrell et al., 2006b). However increasingly, water availability is becoming a constraint to rice production, and year to year variability in planting area is high despite the region having the highest level of water productivity (0.77–1 kg m^{-3}) in the world (Humphreys et al., 2006). This has been particularly so in recent decades with major drought events reducing water allocations, which consequently reduces production area, but also increases the cost of production (Qureshi et al., 2013).

The development of low temperature tolerant rice would provide an opportunity for a significant increase and stabilization of rice yield directly and indirectly by potentially enabling production under reduced water input. One such method for reducing water input is the use of aerobic cultivation, which has been defined by Kato and Katsura (2014) as dry-seeded rice cultivation under non-flooded, non-puddled conditions in which the crop receives irrigation with high levels of fluctuating soil moisture content such that the crop does not encounter any drought limitations.

However, most Australian varieties have been developed for fully flooded irrigation conditions, and varieties suitable for high but reduced water conditions may be required. It could be expected that the effects of low temperature induced sterility might be amplified even further under aerobic conditions, as low temperature exposure in shallow water during the microspore stage doubles sterility when compared with deep water (Borrell et al., 1997; Gunawardena et al., 2003b).

Another factor determining low temperature tolerance is the level of plant nutrition, particularly N availability to the plants. Haque (1988) found that the level of low temperature induced spikelet sterility in rice at the reproductive stage increased further under high N conditions (i.e. in response to higher application of N fertilizer). Under low temperature conditions at the booting stage, the application of high N rates increases spikelet sterility induced by increasing tillering and spikelet numbers per plant, leading to a reduction in the number of engorged pollen grains per anther (Gunawardena et al., 2003a). Thus, spikelet sterility, which is often associated with pollen number per anther is determined not only by cold but also sink-source relationship, which may be altered by water and N nutrition.

While genotypic consistency in tolerance to cold at different development stages is known (Ye et al., 2009), genotypic consistency in cold tolerance under different water and soil nutrition conditions is not known. The objectives of these glasshouse experiments were to identify genotypic variation for tolerance to low temperature in rice, using genotypes derived from a population developed from a cross between low temperature tolerant and susceptible parents, and to determine the genotypic consistency in spikelet sterility between flooded and non-flooded conditions and under varying levels of nitrogen application, when exposed to low temperature during the booting stage.

2. Materials and methods

Three experiments were conducted in two rooms of a controlled temperature glasshouse facility at The University of Queensland, Brisbane, Australia (27°23′S, 153°06′E). In all experiments, plants were grown in a warm glasshouse room maintained at 28/19 °C (day/night) except for 14 days during booting stage under cold treatment where the plants were shifted to a cool temperature glasshouse room; 21.5/13.9 °C (day/night) in Experiment 1, 20.9/13.9 °C (day/night) in Experiment 2, and 23.1/14.8 °C (day/night) in Experiment 3. After the cold treatment period, the plants were returned to the warm glasshouse room. These glasshouse rooms were located next to each other, and other than temperature, growing conditions were identical.

2.1. Genetic material

Norin PL8 was selected as the cold tolerant donor and Kyeema, a current commercial variety, was selected as the recurrent parent. Kyeema (Pelde//Della/Kulu) is an Australian fragrant long-grain variety (considered by industry as susceptible to low temperature) developed by the Australian rice breeding program at the Yanco Agricultural Institute in the Riverina area of NSW, Australia (Troldahl et al., 2014). Norin PL8 is a low temperature tolerant Japanese parental line publicly available for crossing, here considered as a variety (Saito et al., 1995) whose initial cold tolerance was derived from the Indonesian variety Silewah. This study utilised 101 F6, Kyeema//Kyeema/Norin PL8, RIL population developed through Single Seed Descent form BC2F2 head selection, taken by the breeders. A backcrossed population was employed as it was more likely to generate progeny of merit to the breeding program, whose breeding objective in this case was a cold tolerant fragrant long grain replacement for Kyeema. Included in the study were Kyeema, Norin PL8 as well as an Australian low temperature tolerant variety, Sherpa.

Experiment 1 utilised 104 genotypes which included 101 F6 Kyeema//Kyeema/Norin PL8 genotypes, the two parents and Sherpa, and was conducted to select cold tolerant and susceptible genotypes under flooded conditions (Table 1). Experiment 2 and 3 used these contrasting genotypes to examine consistency in cold tolerance by exposing them to cold under different water and soil nutrition conditions. Thus, Experiment 2 was conducted with 20 genotypes (10 most tolerant and 10 most susceptible selected mostly from Experiment 1. The 10 tolerant genotypes included three and the 10 susceptible genotypes included two that were selected from an experiment conducted at the same time under almost identical conditions to Experiment 1) and three varieties, while Experiment 3 utilised 10 genotypes (5 tolerant and 5 susceptible selected from Experiment 2) and three varieties.

2.2. Cultural details, treatments, experimental design and measurements

One litre ANOVApot® (125 mm deep, 125 mm wide at top and 95 mm bottom) which had a central hole in the base of the pot were utilized with an inverted petri dish placed 3–4 cm above the central hole to prevent root escape. Pots were filled with Lockyer prairie soil consisting of light, black clay [USDA Soil Taxonomy: Fluventic Hapludolls; (Isbell, 2002)]. The soil physical and chemical characteristics was described in detail by Powell (1982). Approximately five grams of slow release Osmocote® Pro 3-4M fertiliser (17N-11P-10K-2MgO-TE) was mixed into the soil towards the middle of each pot for Experiment 1 and seven grams of Osmocote was used in Experiment 2, while there was no fertilizer mixed in soil in Experiment 3.

Seeds were directly sown into each pot on 13 August 2013, 6 May 2014 and 22 December 2014 for Experiment 1, 2 and 3 respectively. In Experiment 1, 3 replications were used for all genotypes except that the

Table 1
The growing conditions and timing (days) of low temperature exposure in relation to heading under warm conditions in the three experiments.

Experiment	Genotypes	Water condition	Nitrogen application *(kg/ha)*	Timing of low temperature exposure *(days before heading in warm temperatures)*
1	104	Flooded		4–9 (late booting)
2	23	Flooded and Non-flooded		18 (early booting)
3	13	Flooded and Non-flooded	0 and 150	14–16 (early booting)