A New Diagnostic Tool of Rice Grain Filling and its Response to Stresses using Grain Population Weight and Size Distribution

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Abstract

Abiotic stresses such as salinity affect rice yield components and grain quality. A new, automated methodology is presented to analyze grain weight, length and width distributions for grain samples. Frequency distribution analyses on the basis of histograms generally gave bimodal patterns for grain weight (filled and unfilled grains) and monomodal patterns for grain dimensions. These histograms permit the distinction of unfilled, partially filled and fully filled grains. Peak shape and location on the histogram provide further information potentially useful for the diagnostics of physiological stresses affecting grain hull development, spikelet fertility and filling, and may be of value in breeding and grain quality research. The methodology was applied to rice grain samples taken from farmers' fields having different levels of soil salinity in the Camargue delta region in France. High salinity levels were associated with an increased fraction of unfilled spikelets and reduced grain dimensions and weight, which point at salinity affects taking place largely before flowering during hull development. The methodology is being completed with a biometric tool for histogram analysis, and will be extended to other stresses and germplasm.

Media summary

Distribution analyses of individual grain weight and dimensions in the form of histograms reveal the effects of stresses on yield components, as well as genotypic ability to produce uniform grain through sink regulation. The methodology can potentially be used as a diagnostic tool.

Key Words

Oryza sativa L., spikelet sterility, rice grain quality, source-sink relationships, salinity.

Introduction

Rice is known for its relatively constant 1000-grain weight irrespective of environment, thought to be a result of (1) a rigid hull limiting grain size and (2) variable proportions of spikelet sterility that are apparently regulated according to available assimilates (Yoshida, 1981). Earlier research on irrigated rice in the Sahel documented the extreme sensitivity of spikelet sterility to low temperatures and heat (Dingkuhn et al., 1995) and to a lesser extent to salinity (Zaibunnisa et al., 2002)., but also indicated effects on mean filled grain weight. In fact, histograms of individual grain weight at maturity taken from a population of panicles showed that maximal grain weight decreases under heat stress, whereas a large proportion of grains is only partially filled under cool conditions (Ciss?, 1994). Furthermore, Siband (unpublished, IRRI, 2000) using the same methodology found that in certain rice genotypes, assimilate source limitations caused by leaf pruning lead to highly variable (unregulated) grain weight, whereas in other genotypes the histogram remains divided into two sharp peaks (filled and unfilled: regulating types). A previously unpublished example using a heterotic hybrid (regulating) and a breeding line (poorly regulating) of IRRI is shown in Fig. 1. It was concluded that the degree of bimodality and the shape and position of the peaks on the frequency vs weight histogram may be of diagnostic value for stress effects and genotypic behaviour.

This paper introduces an improved, automated methodology to establish histograms of weight, length and width distributions for grain populations, as well as first results obtained with irrigated rice in the Camargue region in France exposed to different levels of salinity in farmers' fields.

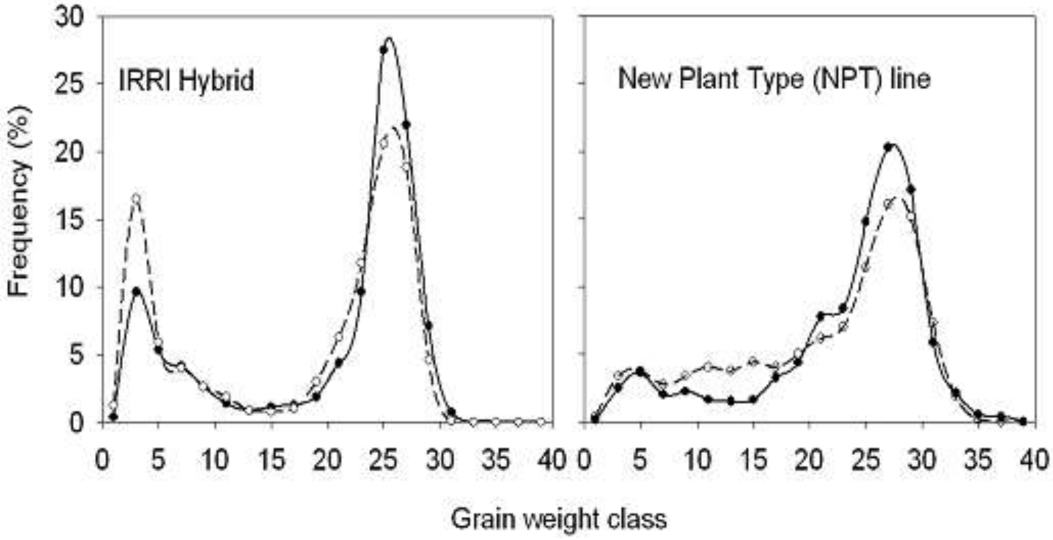

Fig. 1. Example of grain weight histograms obtained for 2 contrasting breeding lines at IRRI; growth without constraints (solid lines) and with leaf pruning at flowering (broken).

Methods

A combined methodology was developed to measure the individual weight, length and width for a population of rice grains in order to study the distribution of these parameters. The method was then applied to grain samples (cv. Ariete; oven dried rough rice including unfilled spikelets) taken from 6 farmers' fields in the Camargue delta region (France) in summer 2003. The fields were known for having different levels of soil salinity, and this was quantified with measurements of soil solution electric conductivity (EC) measured for 0-20 cm soil core samples taken at crop maturity at exactly the locations where panicles were collected. Ten samples of dry soil were extracted with 100 ml water for EC measurement. Mean field EC was between 284 and 981 ?S.cm^{-1}.

Five sub-samples of 50 panicles each were taken in different places from each field. Panicles were threshed by hand and a representative aliquot sample of 20 g (ca. 600 grains) was taken for analysis from each sub-sample. Individual grain weight distribution was measured with a combination of a custom made grain separator using a vibrating bowl and a Precisa XT 220-A precision balance having a resolution of 0.1 mg (grain weight varied between 4 mg for empty grains and 40 mg). The system was controlled by a computer that also served as data logger (Fig. 2). Grain width and length were measured with digital photography of grain samples randomly scattered, followed by processing with SigmaSca- Pro V. 5 software (spherical correction, contrasting and automatic measurement) (Fig. 3).

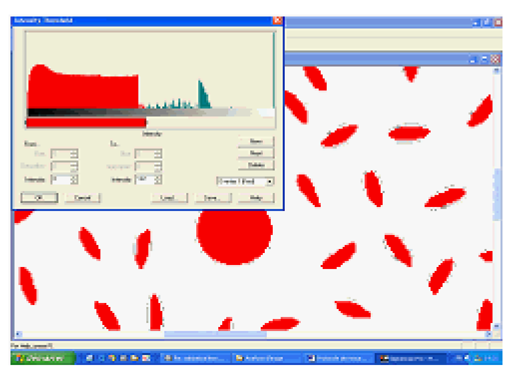

Fig. 2. Device measuring grain weight distribution **Fig. 3. Image analysis of grain dimensions**

Results and discussion

Grain weight histograms revealed three marked differences between the most and the least saline fields (Fig. 4): an increase in the fraction of unfilled grains (left peak), a decrease of mean filled grain weight (right peak) and an increase of the fraction of partially filled grains (weight classes situated between the two peaks). The unfilled grain peak is frequently interpreted as representing sterility, but no distinction could be made between true spikelet sterility and very early cessation of filling.

These observations were consistent across salinity levels, resulting in a positive, linear correlation between the estimated fraction of empty grains and EC (Fig. 5a), and a negative correlation between median filled grain weight and EC (Fig. 5b).

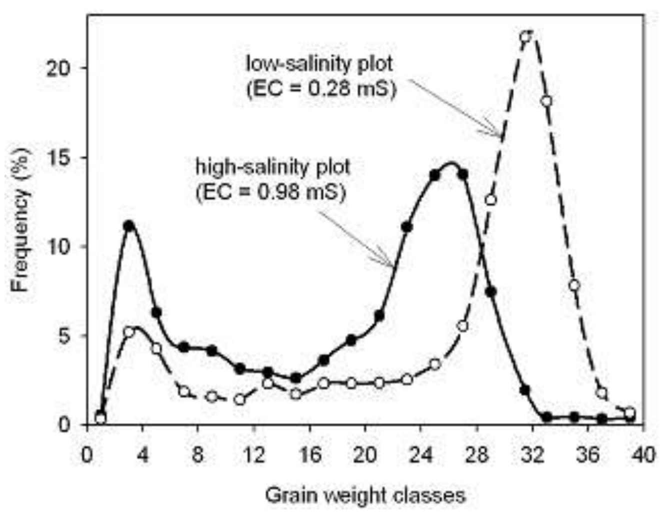

Fig. 4. Frequency distribution of grain weight for rice in a low and a high salinity plot in the Camargue.

Histograms of grain length and width were monomodal, indicating that empty spikelets did not differ in dimensions from filled grains (data not presented). Mean grain width and length decreased linearly with increasing EC (Fig. 6). In summary, salinity was associated with increased fraction of unfilled grains, lower filled grain weight and reduced grain dimensions. These results are consistent with unpublished results obtained at the same sites in the previous year (2002).

It is likely that salinity effects on grain weight were actually brought about by reduced hull size, and therefore took place already before flowering. Increased fraction of empty grains, however, might be due to both "true" sterility (determined before or at flowering) and very early cessation of filling (after flowering). Increased number of incompletely filled grains might be a result of assimilate shortage during grain filling, brought about by early leaf senescence (Sheehy et al., 2001; Murchie et al., 2002) caused in this case by salinity (Shannon, 1998; Zeng, 2000). Drought during grain filling is also known to cause incomplete filling associated with reduced specific weight of kernels (Tsuda, 1993).

The present results demonstrate that histograms of grain weight and dimensions are a potentially powerful tool to analyze how and at what developmental stage stresses affect yield components of rice, although the data presented on salinity effects are at this point fragmentary and require further research. The methodology might be useful for agronomic and physiological crop diagnostics, as well as for characterization of genotypes in breeding. Lastly, it would be of interest to study the relationship between the phenomena reported here and more classical descriptors of rice grain and seed quality. The methodology is currently being completed with a biometric tool enabling the extraction and quantitative characterization of different peaks on the histograms, enabling the detection of anomalies that might be of specific diagnostic value.

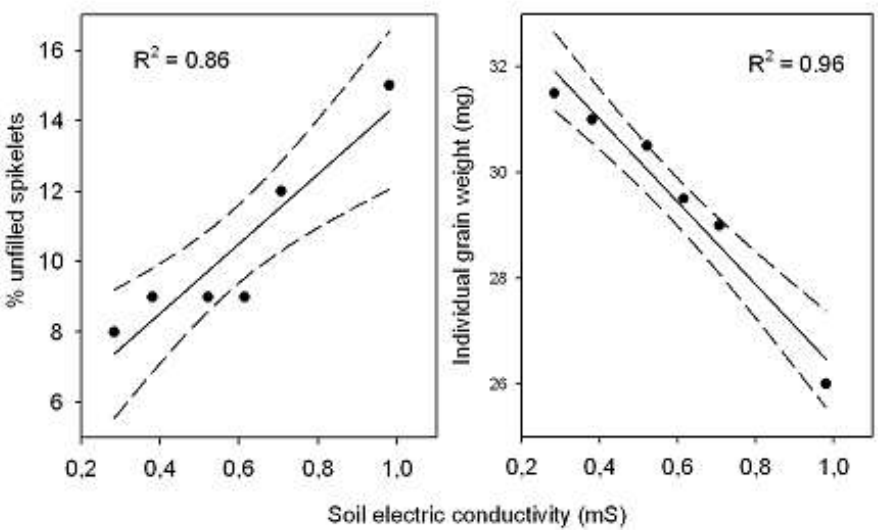

Fig. 5. Relationship between the fraction (%) of unfilled spikelets (left) and individual grain weight (right) with soil electric conductivity (EC).

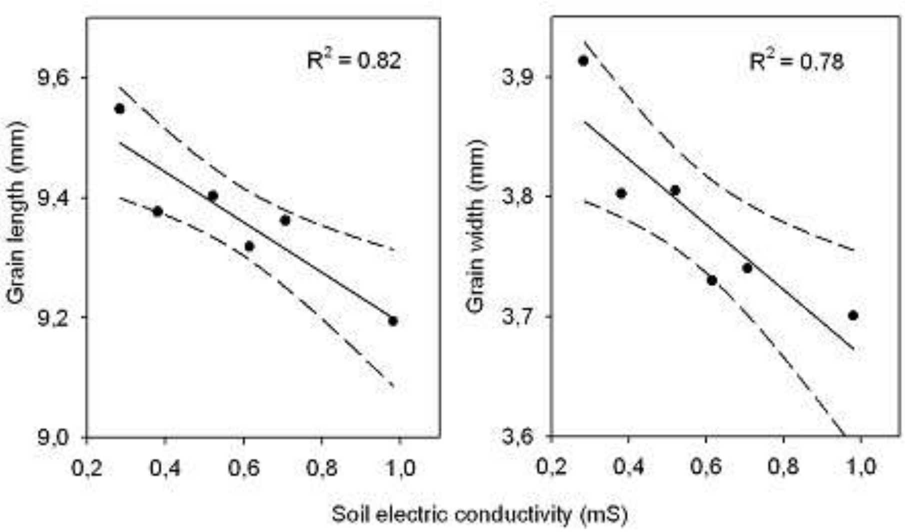

Fig. 6. Relationship between grain lenghth and width with soil electric conductivity (EC).

Conclusion

We presented a new methodology to analyze some yield components of rice related to grain dimensions, sterility and filling. The methodology was applied to salt stressed plants and revealed characteristic effects on yield components. Once these results have been confirmed and interpreted more thoroughly, and the approach applied to other stresses and genotypes, the methodology might be of interest for agronomic diagnostics, breeding, and grain and seed quality evaluation.

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