

Root Screening of a Panel of Rice Accessions

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Abstract

Rice is considered one of the most important cereal crops for human nutrition. Based on current predictions of increasing global population, rice production will need to increase by more than 50% to meet the future demand of the rice eating countries. It has already been demonstrated that cereal crop yield could be increased by modifying root architectural traits for production under limited resources like water and nutrients. The current study was carried out to identify deep rooted cultivars of a panel from *aus* panel from IRRI and a panel consisting of Brazilian and Japanese cultivars by using the buried herbicide technique. Among the accessions of *aus* rice cultivars Narikel Jhupi, Panniti showed the highest herbicide score (HS), 11 cultivars showed the higher herbicide score than the Black Gora (more than 4 HS), 14 accessions showed the same herbicide score as deep rooted check varieties Black Gora, 11 cultivars showed very low herbicide score (less than 0.5) compared to other cultivars and 21 cultivars did not show any herbicide injury symptoms. Among the Brazilian and Japanese accessions IAC 202, Santa America (IAC), Vermelho de Pernambuco, Nourin 5, Urasan 1, Saitama Senshou, Ootama, and Chioda Wase showed the highest herbicide score (more than 4 HS) than the other cultivars. A total of 29 accessions showed very low herbicide score (below 1). Among the all tested cultivars Araure 4, BRS Formoso, Centauro, Cimmaron, CT 9682, Eika Ine, FL00447, Fonaiap, IAC120, IR 8, IRGA 409, Iwate Ryoan 1, Koshihikari, Kurohige, Linea 17 and Nipponbare did not show any symptoms even at the last scoring day. Around 211 cultivars showed less than 3 herbicide score. Screening the root traits (mostly rooting depth), these traits are relevant to breeding of drought resistance in rice. These findings have the potential to be applicable in other field crops as well as rice.

Keywords

Rice, Root, Cultivars, Herbicide, Score, Traits, Accession

1. Introduction

A structured root system is necessary for favourable plant growth. The reason for adopting many cultivation practices is to improve the root environment like soil pH and the drainage system [1]. Crop growth, yield and product quality depends on the roots to a large extent [2]. Absorption of water and nutrients are the principle function of the root and drought is a foremost abiotic stress that affects more than twenty percent of the total rice producing area in Asia [3].

The *aus* group is characterized by a high level of both genetic and phenotypic diversity. The majority of *aus* accessions are considered to be mostly from South and West Asia [4] and they are traditionally grown as short summer season crops under rain fed condition [5]. The *aus* group are grown in extremely diverse agro-ecological conditions. They are grown on hilltops in rain fed lowlands in flood prone and deep water areas and well drained or poorly drained soil and are the source of salt and drought tolerance [6] [7]. These *aus* accessions are generally tall and produce many tillers with short, slender grain in a variety of colour [8] [9].

A recent analysis suggested that, among the 409 diverse accessions, the *aus* accessions originate from Bangladesh and India [10]. The genetic diversity of the *aus* accessions is large and includes cultivars known for their adaptation to different challenging environment. The *aus* cultivars have been recognised as a valuable source of tolerance genes [11]. Different studies suggested that N22 is a heat tolerant cultivar [12] [13] (Yoshida *et al.*, 1981; Ziska and Manalo, 1996; Prasad *et al.*, 2006a; Jagadish *et al.*, 2008). The phosphorus deficiency tolerance *Pup1* gene [14] and *Pup1* specific protein kinase gene, phosphorus starvation tolerance gene *PSTOL1* [15] was detected and cloned in Kasalath (an *aus*). Another study identified and clone the submergence tolerant gene *SUB1A* in FR13A (also an *aus*) [16]. It is also evident that *aus* type variety Black Gora is a deep rooted rice variety [1]. In this study, 150 accessions which were selected and sent by International Rice Research Institute (IRRI) were screened.

The rice and bean research centre of the Brazilian agricultural organisation Empresa Brasileira De Pesquisa Agropecuaria (Embrapa) introduce new cultivars every year to address the demand for improved high yielding varieties which are tolerant to biotic and abiotic stress. Brondani and his co-scientist investigated 192 Brazilian rice landrace accessions using 12 SSR markers [17]. Based on this SSR genotyping result the author recommends that 24 accessions to increase the genetic variability available for selection for breeding.

Breeding of upland rice in japan had been carried out using Japanese upland rice varieties as genetic resources. However, genetic diversity may have already reached a limit for further improvement of the resistance to drought. Nemoto used the trench method to evaluate the root characteristics of 255 rice cultivars. In this method deep rooted rice cultivars were detected through the observation of root characteristics [18]. Till now a significant number of root phenotyping methods were developed by breeders and agronomist to study root traits [19].

In the present study, using herbicide method, a panel of *aus* and Brazilian and

Japanese cultivars were used to discriminate rooting depth amongst cultivars in large collections and hopefully identify deep rooted cultivars.

2. Materials and Methods

2.1. Plant Materials

The *aus* panel were 150 cultivars selected and sent by the International Rice Research Institute (IRRI) and while a panel of 353 cultivars from Brazil and Japan were sent from Sao Paulo University. Both panels were assessed by buried herbicide method [1]. In both screening four cultivars Azucena, Bala, Black Gora and IR 64 were used as check varieties.

2.2. Experimental Set up

2.2.1. Screening of *aus* Panel

The experiment was set up in greenhouse at the end of spring and the beginning of the summer season. The experiment had a randomized complete block design with four replications. The experimental box was set up linearly along the length of the box set up in the greenhouse. A box of 200 cm length, 81.5 cm width and 35 cm depth was positioned on the bench. The box was prepared with plywood wrapped with clean plastic. Non-woven fabric which was folded up the sides of the 5 cm was used to cover the bottom of the box. The box was filled up with soil (mixture of builder's sand 50% and top soil 50%, verve). Theta probes were placed at 15 cm and 25 cm (at the herbicide layer) to measure the soil moisture content throughout the experiment. Diuron herbicide at 100 mg per plant was buried at 25 cm depth using the method of herbicide application [1]. Full strength Yoshida's nutrient solution [20] was applied to saturate the soil of the box. Nutrient solution was applied once throughout the experiment. Seeds were directly sown at the 1.5 cm depth using 5 × 5 cm spacing. Water was applied to germinate the seeds, and after germination watering was stopped. The growing of rice plants in the greenhouse and score of the herbicide injury symptoms scale from 0 to 5 were recorded [1] Where 0 indicates no symptoms, 1 indicates 5% to 15% leaf area affected, 2 indicates substantial leaf 15% - 50% leaf area affected, 3 indicates >50% leaf injury and noticeable leaf death, 4 indicates 15% - 50% leaf area dead, 5 indicates virtual to complete plant death (>50% leaf area). **Figure 1** demonstrates the steps of the experimental set up. The herbicide shoot injury symptoms first appeared at day 20 after sowing and were recorded approximately every other day until 47 days after sowing (DAS).

2.2.2. Screening of Brazilian/Japanese Panel

The experiment was conducted in the same greenhouse during the monsoon in the same condition as maintained in the previous experiment. The experiment was designed to use a similar methodology described as above and applying a randomized complete block design with four replications. The box dimension was bigger due to bigger population size, being 460 cm long with the same width and depth. Diuron herbicide was prepared according to each plant having 100 mg of diuron. Approximately 20 pieces of filter paper (90 cm × 23 cm) were saturated

with diuron herbicide was placed on same depth mentioned in the previous methodology part. Mixture of top soil and builder's sand (50%, 50%) were used in this experiment. Full strength Yoshida's nutrient solution [20] was applied to saturate the soil of the box. After sowing the seeds, water was applied to germinate the seeds, after germination watering was stopped. The other procedure was as the same the previous set up. The score of visible leaf injury was taken six times depending on the symptoms development (Figure 2).

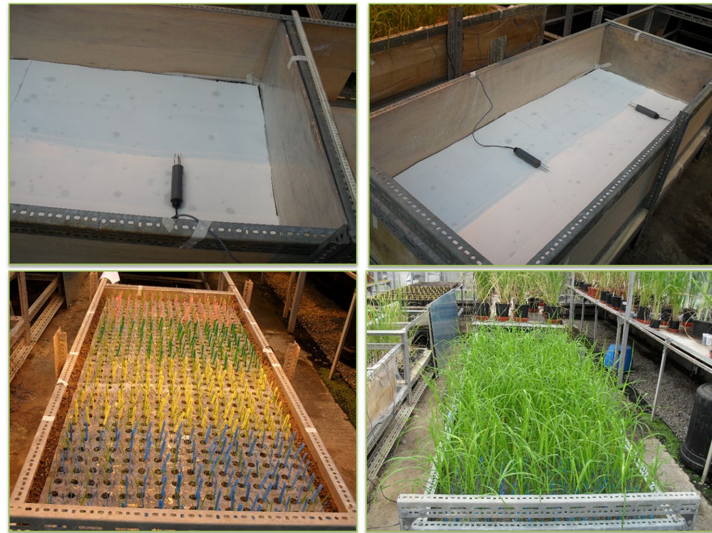


Figure 1. Experimental set up of root screening of *aus* rice cultivars. Diuron impregnated filter paper was laid above the saturated soil and above the filter paper two theta probe were placed (top left and right). The box was filled with top soil (mixture of soil and sand), perforated plastic sheet containing 2 cm diameter holes for sowing plants at a 5 cm × 5 cm spacing was placed on top showing four replications and relative score of visible leaf symptoms from diuron was recorded throughout the experiment (bottom left and right).

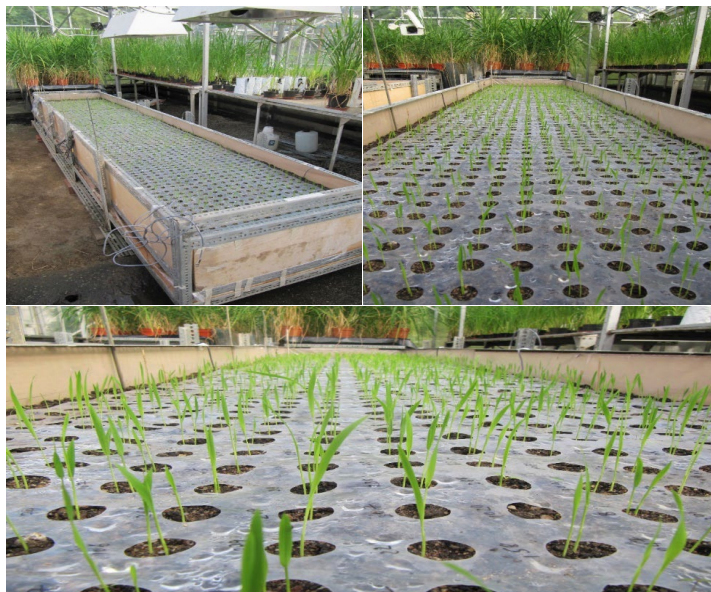


Figure 2. Experimental set up of root screening of Brazilian and Japanese cultivars.

3. Statistical Analysis

One way (ANOVA) using Minitab version 16 was used to carryout analysis of the data. The data were checked for variation due to block by ANOVA or corrected if required. The data were also checked for normality of distribution. For the Brazilian and Japanese accessions screening, there was a strong significant position effect in which there were patches of high or low herbicide symptoms. A spatial correction was applied to the data of % area affected at 34 days by subtracting from the value observed for any plant the mean of the 24 surrounding plants (or fewer plants if it was near an edge).

4. Result

4.1. Soil Water Content and Diuron Toxicity Symptoms Discrimination in the *aus* Panel

The soil moisture content throughout the experiment is presented in **Figure 3**. The soil water content started to reduce in the box at 6 DAS according to theta probe reading. In the herbicide layer, the soil humidity decreased steadily from around 25% but the decrease accelerated from about 17 days to reach 5% by the end of the experiment indicating that plants were taking up water from soil around the herbicide layer.

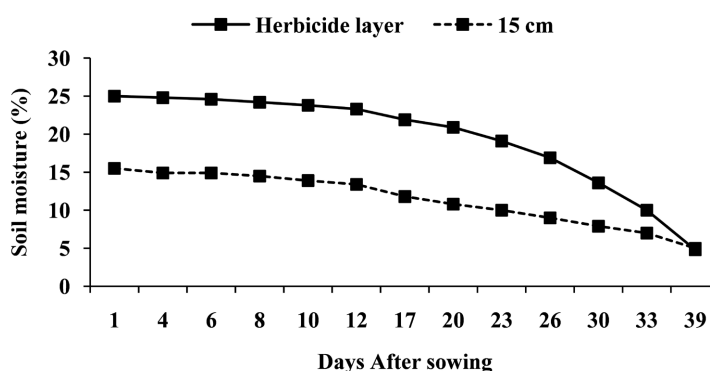


Figure 3. Theta probe readings of *aus* panel of two different depth (herbicide layer and 15 cm of the experimental box, half way of the experiment).

The progression of the mean herbicide score (HS) throughout the experiment is presented in **Figure 4**. The herbicide symptoms were first recorded at day 20 after sowing where 13 plants had developed symptoms. The herbicide injury symptoms were recorded on eighth occasions from day 20 to day 47 after sowing. On each occasions ANOVA shows the proportion of variation explained by genotypes was highly significant ($P < 0.01$) and were in the range of 31.4% to 35.6% of variation explained with F values in the range of 1.5 to 1.8 and which did not change much with time. The highest discrimination between the genotypes were observed in the day 47 after sowing. The frequency distribution of herbicide score on day 47 is displayed on **Figure 5**. The variation of mean herbicide score of the

accessions on day 47 is shown in **Figure 6** and **Figure 7**. Out of four check varieties Black Gora had a mean score 1.75 with Azucena 0.5 on day 47. Bala and IR 64 did not show any symptoms even at the last scoring day after sowing. For 70 cultivars there were no symptoms even after 47 days. For the rest their herbicide score on day 47 are presented in **Figure 6** and **Figure 7**. The mean herbicide score of Azucena and Black Gora during the screen shown in **Figure 8**. Among the accessions Narikel Jhupi (more than 4 HS), Pannitti (More than 3 HS) showed the highest herbicide score compared to the other cultivars. Kele, kalia, Dheki shaita, Dharia boalia, Boteshwar, Noroi, Kalu A 30, Dharia, Koi Murali, Dhali Khama, Baulan showed higher herbicide score than the Black Gora while Tepi Boro, Simul Khuri, Seenetti, Lenza Murali and Gerdeh showed the same score as Black Gora (**Figure 6**). Bathuri, Garia, ARC 12071, Kangro, Bawoi, Gulmurali, Jambali Bussa, Madhabsail 741, N22 and Mahlar 346, Santhi sufaid 207 showed very low score (less than 0.5) even on the last scoring day (**Figure 7**). Anglais, ARC 10317, ARC 10955, *Aus* 196, 257, 41, 439, Bailam, Bans 4, Baranboro, Biranj, Chengri, Chengri Murali, Chiadi Naki, Chikon shoni, DA 16, DA 18, Dangar, Dhal kachi, Dhala bhadoi, Fulkati, they did not show any symptoms even at the end of the experiment.

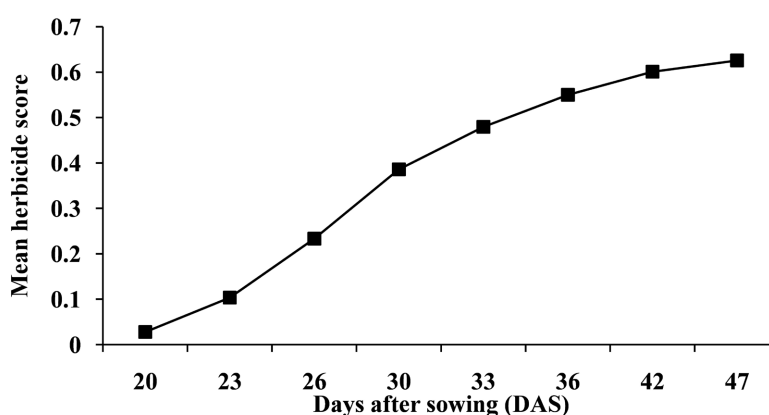


Figure 4. Mean diuron herbicide score of *aus* panel at different days.

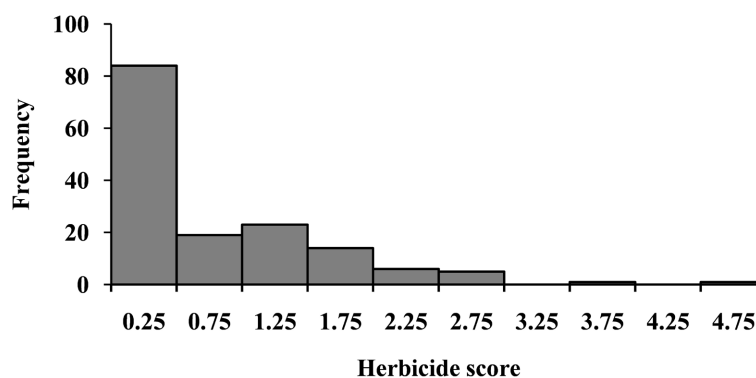


Figure 5. Frequency distribution for herbicide score of *aus* panel at 47 days after sowing.

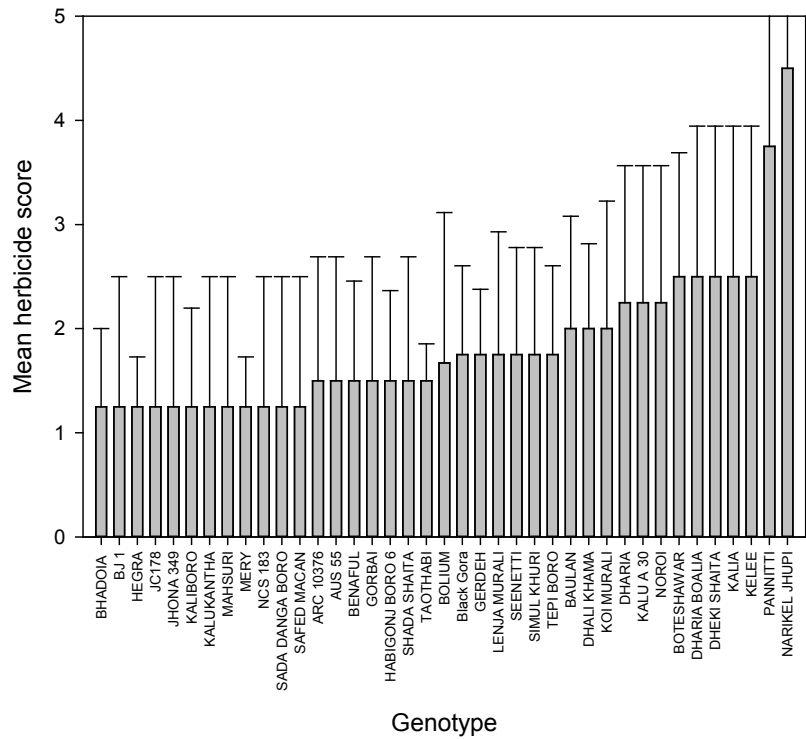


Figure 6. *Aus* Cultivars marked in order of mean herbicide score at day 47. Error bar represents standard error, n = 4. *Aus* cultivars showing herbicide score 1.25 to higher in this figure.

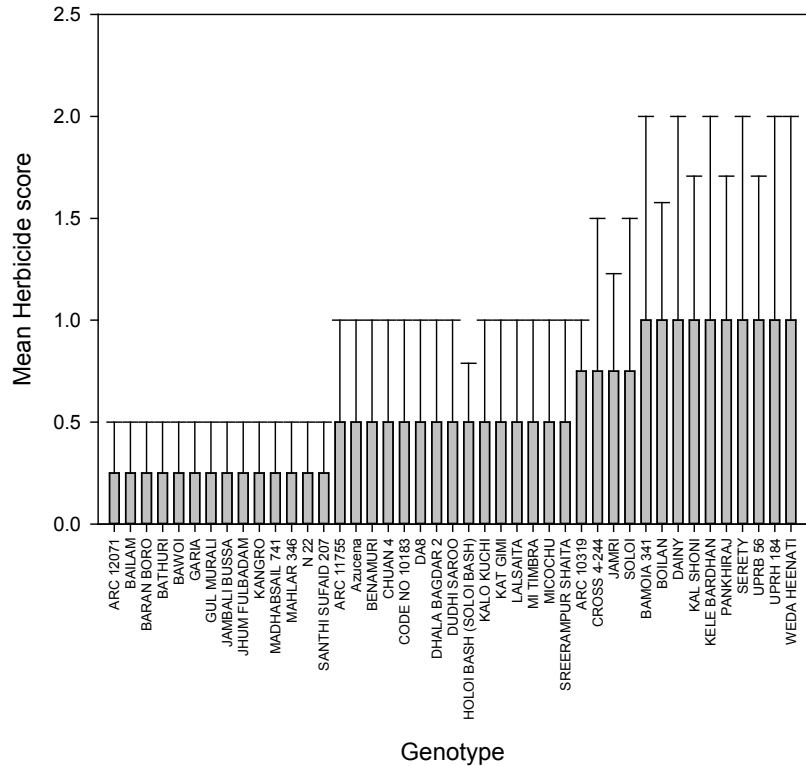


Figure 7. *Aus* ultivars marked in order of mean herbicide score at day 47. Error bar represents standard error, n = 4. Cultivars showing herbicide score below 1.25.

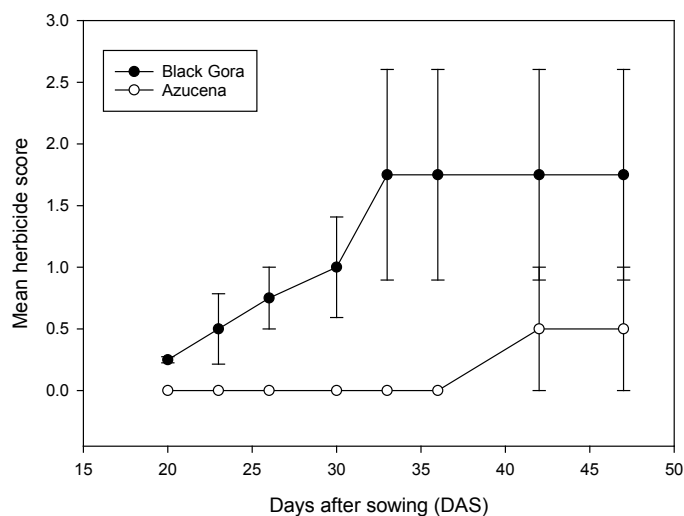


Figure 8. The variation in mean herbicide score of Black Gora and Azucena in *aus* panel from day 20 to day 47 after sowing (error bar = standard error).

4.2. Soil Water Content and Diuron Toxicity Symptoms of Brazilian and Japanese Cultivars

The result of the theta probe readings throughout the experiment are presented in **Figure 9**. The soil water content at the herbicide layer (25 cm) was above 25% at the start of the experiment, after that the moisture level slowly dropped steadily to reach around 10% by the end of the experiment. At 22 days after sowing (DAS) symptoms started on twenty cultivars and data were collected every third day. On the first scoring day cultivars 6-V-7, Aulha Esav, Arabiya Mochi, *Aus* 61, Black Gora, Col/Miyazaki/1963, gaisen Mochi, IPSL 2070, Ishiya Kushi Mochi, Kang youho, Linha 2459, Nourin Mochi 4, Oosumi, Owri 79, Piemonte IAMG N4, Saitama Senshou, Senshou, Senshou Ibaragi, Sonobe Mochi, Touzam Mochi, Vermelho de Pernambuco, Yonoyuki Mochi had plants which showed symptoms. The overall average symptoms were very low at the beginning and rose to near 2.5 at the end of the scoring at 34 days after sowing (**Figure 10**). On each day of measurement, cultivar had a significant effect on herbicide score as detected by one way analysis of variance (ANOVA). The F value from ANOVA increased from day 22 to day 34 (**Figure 11**) and it was highest (1.54) in the 34 DAS. It suggested that this was the day where it showed the most discriminating between the cultivars. At this time the ANOVA indicated that genotype explained 34% (R^2 value) of the variation within the population. The frequency distribution of herbicide score on day 34 is displayed on **Figure 12**. The result revealed that by 34 DAS IAC 202, Santa America (IAC), Vermelho de Pernambuco, Nourin 5, Urasan1, Saitama Senshou, Ootama and Chioda Wase showed the highest herbicide score (more than 4 HS) than the other cultivars (**Figure 13**). These cultivars showed higher herbicide score than the Black Gora, the deep rooted check cultivars [1] [18]. It suggested that those cultivars are deep rooted accessions among the tested cultivars. A total of 29 accessions showed very low herbicide score (below 1) at the end of the scoring day. Among the all tested cultivars Araure 4, BRS Formoso, Centauro, Cimmaron, CT 9682,

Eika Ine, FL00447, Fonaiap, IAC120, IR 8, IRGA 409, Iwate Ryoan 1, Koshihikari, Kurohige, Linea 17 and Nipponbare did not show any symptoms even at the last scoring day. Cultivars which had 3 and more than 3 for herbicide score at day 34 are chosen to show in **Figure 13** and **Figure 14**. Around 211 cultivars showed less than 3 herbicide score. Out of four check varieties Black Gora has shown a maximum score of 4.5 on day 34 after sowing and Azucena has shown maximum 2 on end of the score. The variation of the symptoms development of these two check cultivars throughout the experiment are shown in **Figure 15**. The checks Bala and IR 64 did not show any symptoms throughout the experiment.

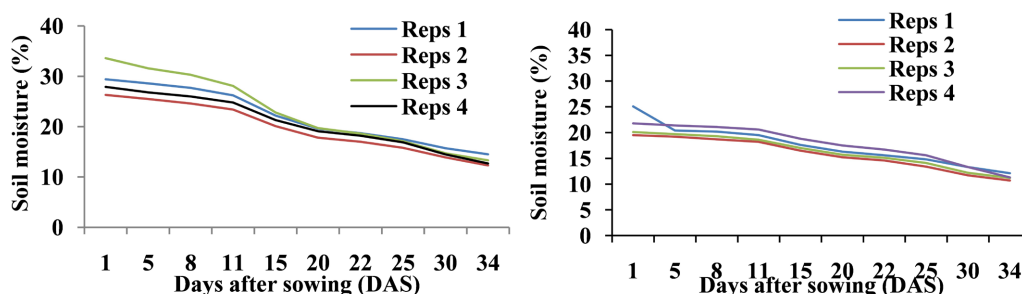


Figure 9. Soil moisture content throughout the experiment of Brazilian and Japanese panel at different days of four replicates at different depth, herbicide layer (left) and 15 cm of the experimental box (right).

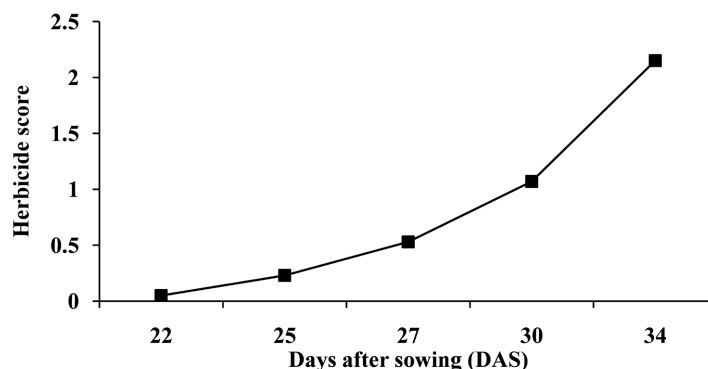


Figure 10. Mean herbicide score of Brazilian and Japanese cultivars at different days after sowing (DAS).

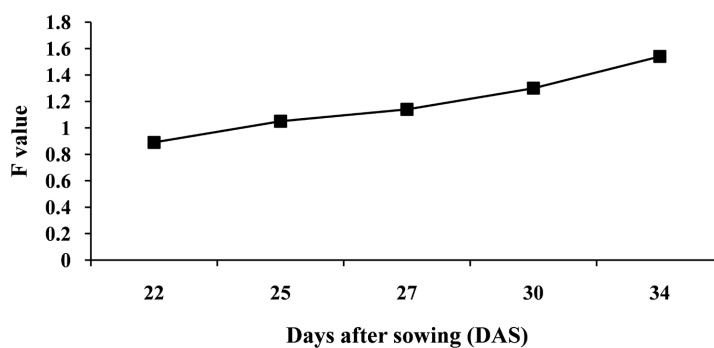


Figure 11. F value of one-way ANOVA for herbicide score with factor genotype of Brazilian and Japanese cultivars at different days after sowing (DAS).

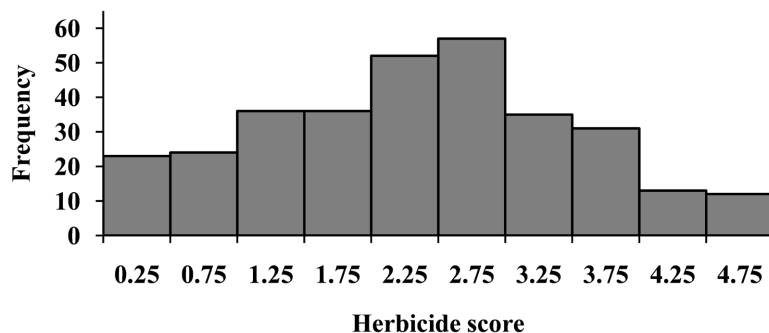


Figure 12. Frequency distribution of Brazilian and Japanese cultivars for herbicide score at 34 days after sowing.

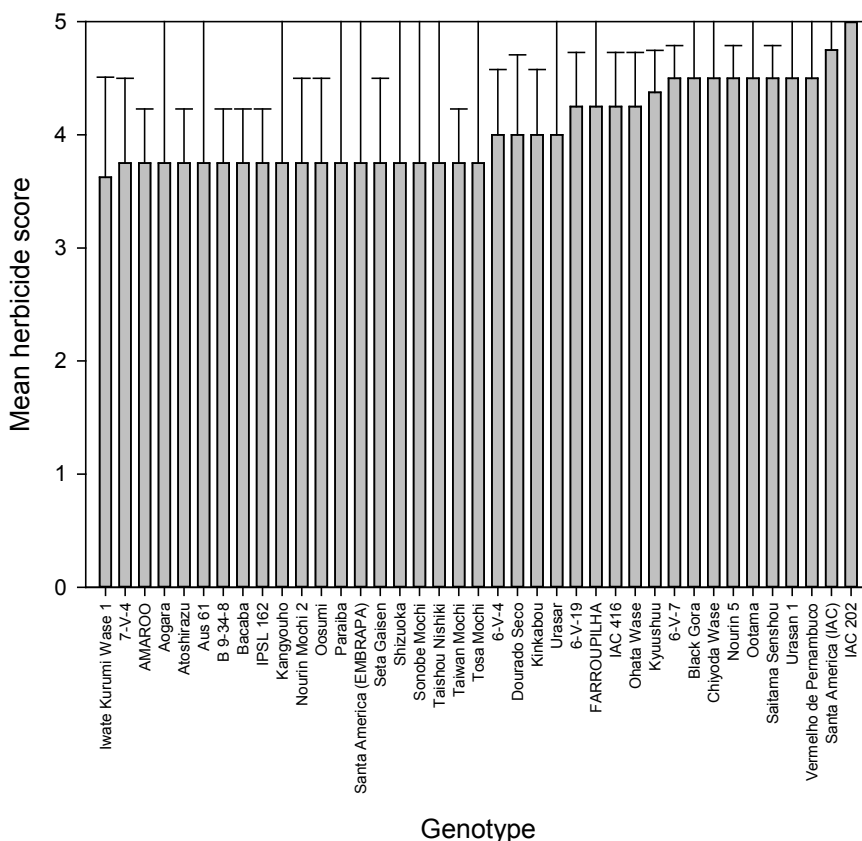


Figure 13. Brazilian and Japanese Cultivars shows mean herbicide score on day 34. Error bar represents standard error mean, n = 4 (cultivars shows herbicide score more than 3).

5. Discussion

The buried herbicide technique used here is a novel method used in root screening of rice described in Al-Shugeairy *et al.* (2014) [1]. The authors suggested that the strong correlation between mean herbicide and the root traits strengthen the confidence about using this method for assessing large numbers of genotypes for rooting depth. The check varieties used in these two experiments have already been established by different experiment with diuron herbicide [1].

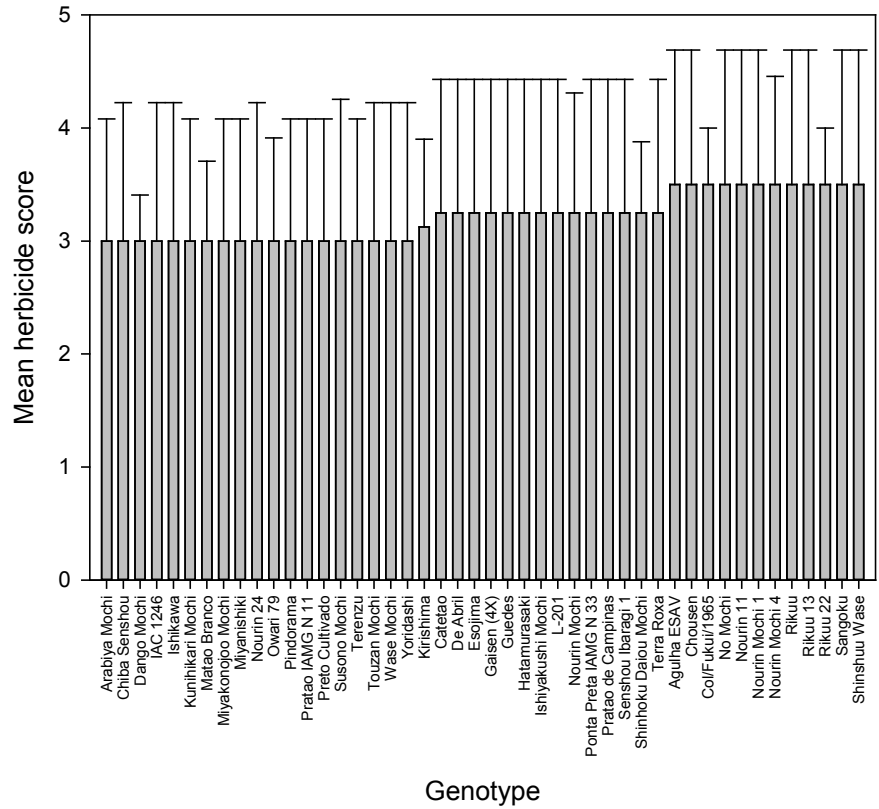


Figure 14. Brazilian and Japanese cultivars show mean herbicide score on day 34. Error bar represents standard error mean, n = 4 (cultivars shows herbicide score 3 and higher).

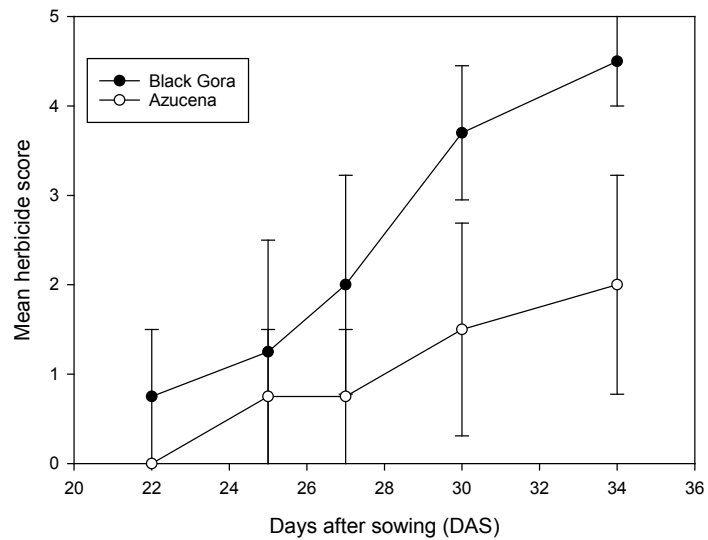


Figure 15. The variation in mean herbicide score of Check Cultivars Black Gora and Azucena from day 22 to day 34 after sowing (error bar = standard error).

Visual observation of the phytotoxicity symptoms is the basis of this method where it can be considered as an effective way of expressing in the above ground shoots the speed with which the root reaches the herbicide layer and absorbs herbicide. In the *aus* panel screening symptoms development was slower than the ex-

pected and did not result in large scale plant death. **Figure 4** showed that herbicide symptoms development surprisingly slow. The experiment with the Brazilian/Japanese panel had much more rapid symptom development. In comparison to previous experiment [1], the mean herbicide score at the end of scoring day 47 (DAS) in the *aus* panel was less than 1 (**Figure 4**) and in Brazilian/Japanese panel at day 34 (DAS) it was less than 3 (**Figure 10**). The check variety (Azucena and Black Gora) also developed symptoms very slowly in the *aus* panel screen (Bala and IR64 did not show any symptoms throughout both screens) in comparison with the speed and severity of symptoms development observed in the previous experiment and the evidence [1]. This observation suggests that the *aus* panel screen was not a great success and may reflect the environmental condition of the glass house. However, the exact factors that caused this variation are unknown.

The previous experimental evidence indicated differences in sensitivity in the accessions will only affect the speed of symptoms development by a few days [1]. It is important to note, therefore, that this method is a positive test for deep rooting but not a negative test for shallow roots [1]. Rapid development in the symptoms must represent rapid root growth, meaning that those cultivars which show early symptoms are deep rooted cultivars. However, low or even no symptoms cannot be used to identify the shallow rooted accessions since these lack of symptoms might represent resistance to herbicide.

6. Conclusion

The buried herbicide technique is a robust method to use the root screen of diverse rice accessions or landraces. Occasionally it does not work to its optimum potential but the reason is as yet unknown. It can, however, be used in large scale screening of the rooting behaviour of rice plants in the early vegetative stage. Here it has been used to show that variation in rooting depth exists in an *aus* and a Brazilian/Japanese panel. In both panels, some potentially deep rooted cultivars have been identified. This trait information can be used for further analysis for the breeding programme specially to breed drought tolerant rice varieties for the drought prone regions of these area. For both these panels other organisations are generating high density genetic data meaning that this root data could be used by them for mapping genetic components that determine cultivar differences in root depth.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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