Developing a buried herbicide method to assess rooting depth of different rice cultivars (*Oryza sativa* L.)

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Abstract:

Rice (*Oryza sativa* L.) is one of the main staple foods of the world. With the increase of population and the deficit of irrigated water, the increase in rice production that is predicted will be dependent on areas prone to drought. Root depth is important for plant growth and survival during drought because of its role in facilitating water uptake from deep soil layers. By advances in genomics, the plant root systems can be linked to quantitative trait locus (QTL) information to achieve a most beneficial design of root system architecture. There is a demand to develop and validate techniques that permit estimation of the root system. Therefore, one technique (a buried TRIK and Diuron herbicide method at depth 30 cm) was used in this study to assess root traits in a total of 32 rice cultivars. The results from these screens was assessed with root traits measured on the same cultivars in the rhizotrons, hydroponic and non-woven fabric experiments. Correlations between these methods showed that herbicide score at day 35 was most strongly related to traits of the rhizotron experiment, especially number of roots passed 50 cm at 35 days, root angle at day 21, root thickness, water use and % root mass. Using all of these traits obtained in the rhizotron in a best subset regression suggests that up to 71% of the variation in herbicide score can be explained. These data strongly imply that symptoms are related to root development and transpiration demand and are therefore ideal for assessing water extraction by roots at depth. Therefore, developing a cost effective high-throughput system that can measure traits related to drought avoidance on a large number of plants would aid genetic studies in breeding.

Key words: Rice, buried herbicide, TRIK, Diuron, (Oryza sativa L.)

***Part of PhD dissertation for the first author.**

تطوير طريقة دفن مبيدات الأعشاب لتقييم صفة عمق الجذور لاصناف مختلفة من الرز

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المستخلص:

الرز هو واحد من الأغذية الأساسية الرئيسية في العالم. مع زيادة السكان والشحه في مياه الري اصبح من الضروري العمل على زيادة إنتاج الرز خصوصا في المناطق المعرضة للجفاف. لذلك تعتبر صفة عمق الجذور مهه لنمو النباتات من اجل بقائها على قيد الحياة أثناء الجفاف بسبب دورها في تسهيل امتصاص المياه من طبقات التربة العميقة. نظرا للتقدم الحاصل في علم الوراثه، وأنظمة جذور النباتات يمكن ربط صفة عمق الجذور بالمواقع الكمية غلى جينوم الرز لتحقيق التصميم الأكثر فائدة للنظام الجذري لامتصاص المياه من اعماق التربة. لذلك اصبح هناك طلب على تطوير تقنية تسمح بتقدير صفة عمق الجذور، تم استخدام أسلوب دفن مبيدات الاعشاب (TRIK and Diuron على عمق 30 سم) لتقييم صفة عمق الجذور الى 32 صنف من الرز. تم تقييم النتائج من هذه الدراسة مع نتائج دراسات اخرى لتقييم هذه الصفة حيث استخدمت نفس الاصناف باستخدام تجارب Rhizotrons و hydroponic و non-woven fabric اظهرت النتائج وجود ارتباط قوي بين تقييم صفة عمق الجذور في يوم 35 بعد الزراعة في تجربة مبيد الاعشاب مع الصفات المدروسة في تجربة rhizotrons خاصة صفة عدد الجذور على عمق 50 سم في بوم 35 بعد الزراعة ، زاوية الجذر في يوم 21 بعد الزراعة ، سمك الجذور، النسبة المئوية لكتلة الجذور واستهلاك الماء في اليوم الثالث بعد الزراعة. تم تحليل هذه الصفات مع صفة عمق الجذور في طريقة دفن المبيد باستخدام تحليل الانحدار best subset regression اشارت النتائج اكثر من % 71 من الاختلافات تعود الى صفة عمق الجذور في تجربة دفن المبيد والتي لها علاقة بتطور نمو الجذور ومتطلبات النتح التي لها علاقة باستخلاص الماء من الاعماق عن طريق الجذور. لذلك تطوير تكنيك فعال لتقييم صفة عمق الجذور لاعداد كبيرة من اصناف الرز التي لها علاقة بتجنب الجفاف في وقت قصير وتكلفة قليلة لها اهمية كبيرة في برامج التربية وتحديد الجينات المسؤولة عن مقاومة الجفاف.

كلمات مفتاحية: **الرز، TRIK، Diuron، مبيد الاعشاب.**

*** جزء من اطروحة دكتوراه للباحث الأول**

1 Introduction:

Root growth is a crucial component for developing drought resistance in rice (12) A rice plant with a deep root which is able to take up water from deep soil layers is desirable in drought resistance and genotypic variation in root system morphology is present in rice (10) In fact, maximum root length and thickness in rice have been shown to be significantly correlated with drought resistance (9) Field drought resistance was related to deep rooting (12,18) noted that difficulty in selecting root growth characteristics on a large scale in the field has so far impeded progress at using root traits to improve drought resistance. Although field screening can give the precise capability of rice roots to penetrate soils in natural situations, it needs hard field work, large fields, is costly and cannot evaluate several rice cultivars at the same period (8).

Several attempts have been used to screen root depth by applying an herbicide method. (14) developed a field technique to screen root depth of 15 cowpea cultivars (*Vigna unguiculata* L.). A preliminary herbicide experiment was carried out in 1980 by inserting a banded strip of Metribuzin (4-Amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5 (4H)-1) herbicide with 5 cm width, 20 m long and 56 cm depth at the centre between two rows of plants. The experiment was irrigated by sprinkler until field capacity and then received no further irrigation. This experiment was repeated in 1982 with some modification by using additional herbicide treatments and two rates of herbicide (4.5 and 9.0 kg per hectare). Another study was done by (5) who concentrated in his trial to screen root growth of 100 diverse cucumbers *(Cucumis sativus* L.) in the greenhouse and in the field by using a subsurface herbicide banding technique. In addition, pot experiments showed that 0.25 or 0.50 kg Simazine (6-chloro-N, N′-diethyl-1, 3, 5-triazine-2, 4-diamine) per hectare produced distinctive symptoms. Grumet and his colleagues conclude that response time and severity of symptoms varied with herbicide concentration, depth, and distance from the seed row. Metribuzin and Simazine were selected because they consistently produced distinctive symptoms in leaves of plant at low concentration. A total of four rice genotypes (IR 36, IRAT 216, Azucena and Vandana) were screened by (16) using a manual injection method with three herbicides (Atrazin, Diuron and Metribuzin) at different depths in the glasshouse and field experiments. The authors detected significant differences in the number of plants affected between genotypes.

There is a challenge to developing high throughput systems that can measure relevant traits on large numbers of plants. (4) noted that, with advances in genomics and the emergence of new techniques that will permit easier and more precise phenotyping of root system architecture (RSA), the patterning of plant root systems can be linked to quantitative trait locus (QTL) information to obtain an optimal design of RSA. Most root QTLs identified so far are small but there are a few examples of QTLs that individually contribute up to 30% of phenotypic variation for root traits in rice (12). A meta-QTL analysis by (3) used 675 rice root QTLs from 12 mapping populations of rice controlling 29 root parameters (including root number, maximum root length, root thickness, root/shoot ratio and root penetration index). Courtois' study summarises a huge amount of historical work on root QTLs in rice which hold promise for breeders. A major limitation in the use of genetic mapping to study the root system is the requirement to test a large number of plants, hence a high-throughput system for root screening would be a major advance.

This study tests if it is possible to screen root depth in rice in the greenhouse by burying TRIK or Diuron herbicides at 30 cm depth in soil by way of affirming the observations of (7) who suggested that herbicide symptoms on wheat were a result of roots reaching the depth where the herbicide was buried. The experiments examined if TRIK and Diuron herbicides were any different in their phytotoxicity and usefulness as a chosen agent. In order to obtain confidence that herbicide symptoms were related to root traits, correlation analysis was conducted between root traits measured on the same varieties using three other methods. The outcome from these studies would allow testing of root depth in large genetically characterised populations in later chapters of this thesis such as recombinant inbred lines of the Bala x Azucena cross and a Rice Diversity Panel developed for association mapping. Therefore the main objectives of this study were to:

1. Investigate if cultivars differ in the speed with which they develop symptoms of herbicide damage when exposed to either herbicides buried at 30 cm depth in soil.
2. Investigate if the symptoms observed can be related to variation in root traits measured on the same cultivars using three other methods of varying simplicity; large glass fronted rhizotrons, hydroponics and non-woven fabric penetration test. Correlations with root traits would provide confidence that this method is useful for screening the root systems of rice.
3. Compare the utility of two different herbicides TRIK and Diuron.

2 Materials and Methods:

2.1 The effect of buried TRIK herbicide on 32 different rice cultivars (*Oryza sativa* L.)

2.1.1 TRIK herbicide used

TRIK herbicide (active components as 46.7% w/w Diuron, 28 % w/w Aminotriazole and 14% w/w 2,4-D) (Figure 1) manufactured by Nufarm UK limited, was purchased from commercial sources in UK many years ago. Each component has a unique mode of action. Diuron acts by inhibiting the Hill reaction in photosynthesis to hinder CO_2 fixation and the production of adenosine triphosphate (ATP). Aminotriazole inhibits imidazole glycerol phosphate dehydratase activity which is involved in the plant histidine biosynthetic pathway (most likely localized within chloroplasts) and catalyzes a dehydration reaction to produce imidazole acetol phosphate from imidazole glycerol phosphate (12). 2, 4-D is a synthetic auxin, a class of plant hormones. It causes uncontrolled, unsustainable growth causing stem curl-over, leaf withering, and ultimate plant death. In this study the TRIK herbicide dose was calculated based on previous experiments conducted by Roshi Shrestha where 100 mg per plant proved effective.

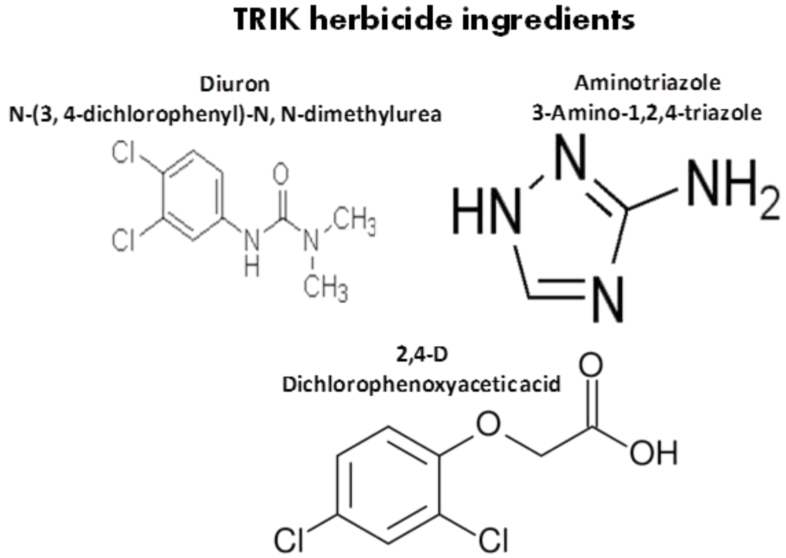

Figure 1: TRIK herbicide consists of three ingredients Diuron, Aminotriazole, and 2, 4-D.

2.1.2 Preparation of experimental soil boxes

A total of four clear plastic boxes (length 52.5, width 32 and depth 39 cm) containing loam top soil from Rolawn Ltd (UK) and herbicide were prepared as follows. The top soil used had a volumetric water content of 18.5%. To this 125 ml of nutrient solution per litre of soil was added to reach 27% water content. First, a 5 cm top soil layer saturated with Yoshida's full strength nutrient solution (18) was placed at the bottom. Above this was placed a filter paper soaked with herbicide. Each plastic box had its filter paper soaked with 3.2 g herbicide which was applied by soaking the filter paper (length 50 and width 30 cm) with TRIK herbicide in 41 ml water followed by air drying (Figure 2). A total of 28 litres of soil was place in each box above the filter paper, filling it to a depth of 15 cm depth. Then to measure soil water content, a theta probe (Delta T Devices, UK) which had soil packed quite tightly inside the prongs was placed gently on top. After that another 28 litres of top soil was added to fill until 30 cm depth. A total of 7 litres of nutrient solution was prepared then added slowly, one third at a time which ensured that the water was slowly percolated into the soil avoiding mass flow of water that would have promoted soil movement. Theta probe readings were taken before, during and after watering. On top of the soil, plastic sheets (52.5 x 32 cm) were placed on the soil surface, the plastic sheets containing 32 perforations (2 cm diameter) for sowing rice plants at a depth 1.5 cm using a 5 x 5 cm spacing (Figure 3(A)). Each box was surrounded by plastic sheeting, white and black side, white side uppermost prevent light entry (Figure 3 (B)).

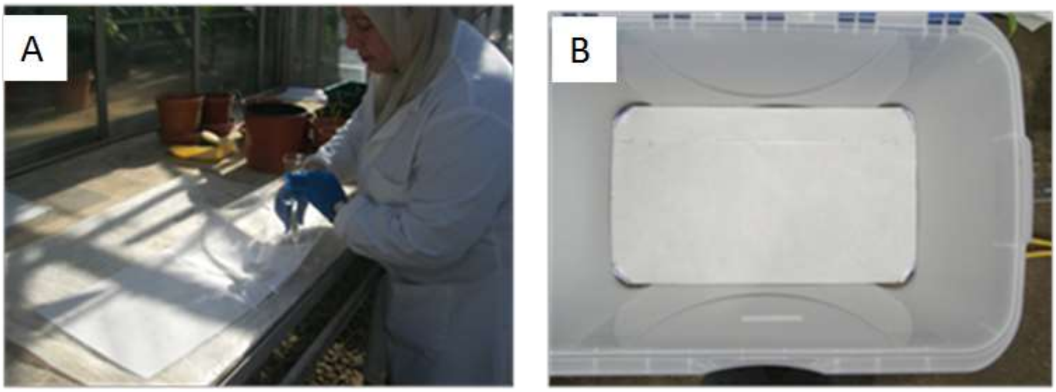

Figure 2: Inserting the herbicide: (A) Strip of filter paper is saturated with 3.2g TRIK herbicide in 41 ml water and (B) Filter paper soaked in TRIK herbicide was laid above a 5 cm thick saturated soil layer.

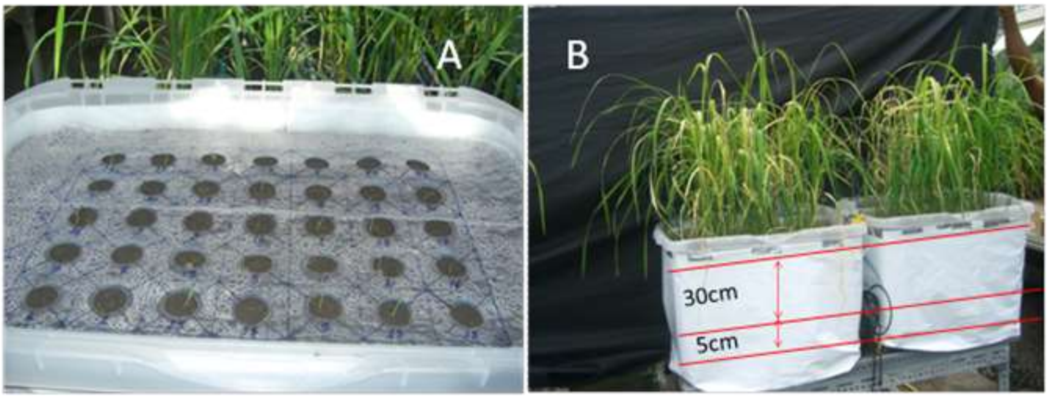

Figure 3: (A) Pre-germinated cultivars were sown one in each hole and (B) experiment design placed filter paper saturated with TRIK herbicide above 5 cm top soil saturated with Yoshida's nutrient and filled to 30 cm with top soil.

2.1.3 Rice seeds utilized

A total of 32 different rice cultivars (Akihikari, Aswina, Azucena, Bala, Black Gora, CT 9993, Cypress, Dom Sufid, Dular, FR 13A, IAC 165, IAC25, IR 62266, IR 64-21, Kinandang Patong, Labelle, Lemont, Li-Jiang-Xin-Tuan-Hei-Gu, M 202, Minghui 63,

Moroberekan, N 22, Nipponbare, P F21-01-03-11-07Y, PYF3-26-5-18, Rayada, Sadu Cho, Sanhuangzhan No 2, SHAI-KUH, Swarna, Tainung 67and Zhenshan 97) were used in this experiment which obtained from IRRI.

2.1.4 Growing rice plants in the greenhouse

A total of 32 different rice cultivars were grown in the Cruickshank greenhouse which has a minimum temperature of 25^{o}C. Supplementary light of 150 µmol m^{-2} s^{-1} PAR was provided for 12 hours a day. Pre-germinated seeds were placed at the depth of 1.5 cm in the top soil (Figure 3).

2.1.5 Setting up the experiment and scoring affected leaf area.

The design of this experiment was a randomized complete block with four replications. The herbicide symptoms are normally visible in new leaves as chlorosis concentrated around veins followed by necrosis. Two weeks after sowing, a relative score of visible leaves symptoms from TRIK herbicide was recorded weekly, where 0 indicates no symptoms (Figure 4 (A)), score 1 indicates noticeable leaf yellowing (5-15% of leaf rea affected) (Figure 3.4 (B)), score 2 indicates substantial leaf yellowing (15-50% leaf area affected) (Figure 4 (C)), score 3 indicates substantial leaf yellowing (>50% leaf area affected) and noticeable leaf death (5-15% of leaf area) (Figure 5 (D)), score 4 indicates substantial leaf death (15-50% leaf area dead) (Figure 4 (E)) and score 5 indicates virtual to complete plant death (>50% leaf area) (Figure 4 (F)). Theta probe readings were taken regularly. The humidity started to decrease in the boxes at day 16 after sowing (DAS). Water was added with the aim of keeping the soil water content at 25%. Plant height was measured weekly.

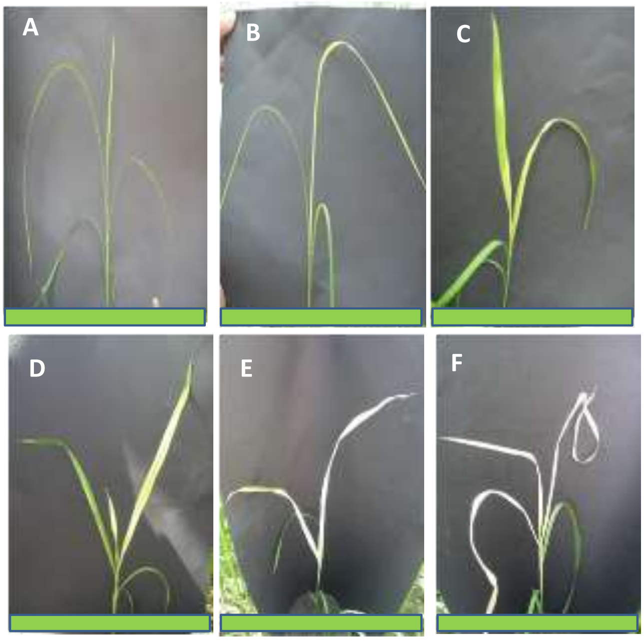

Figure 4: (A) Score 0 (no symptom); (B) Score 1 (5 – 15% of leaf affected); (C) Score 2 (15 – 50% leaf area affected); (D) Score 3 (> 50% leaf area affected); (E) Score 4 (15 – 50% leaf area dead); (F) Score 5 (> 50% leaf area dead).

2.2 Comparing TRIK and Diuron herbicides

Four boxes constructed similarly to those above were used to compare the effect of TRIK and Diuron herbicides. There were two boxes for each herbicide and each box contained two randomly arranged replicate plants of 17 different rice genotypes IR 64-21, Moroberekan, FR 13A, M 202, Black Gora, Bala, Nipponbare, Kinandang Patong, Zhenshan 97, Dom Sufid, Dular, Azucena, N22, Rayada, RIL4, RIL104 and RIL21. The rice plants were sown in a controlled environment growth room on the 8th December 2010. Supplementary light of 150 µmol m^{-2} s^{-1} PAR was supplied for 12 hours a day within temperature range from 28 - 30°C (Figure 5). To determine the dose for Diuron herbicide, calculation was done based on the percentage composition of Diuron in TRIK (46.7% mg) ensuring that the treatments received the same Diuron dose (e.g., they were given 1.58 g Diuron per box, or 46.7 mg per plant). Because Diuron is not soluble in water, it was dissolved in 100% ethanol before soaking onto the filter papers. All ethanol was dried off before the filter paper was put in place.

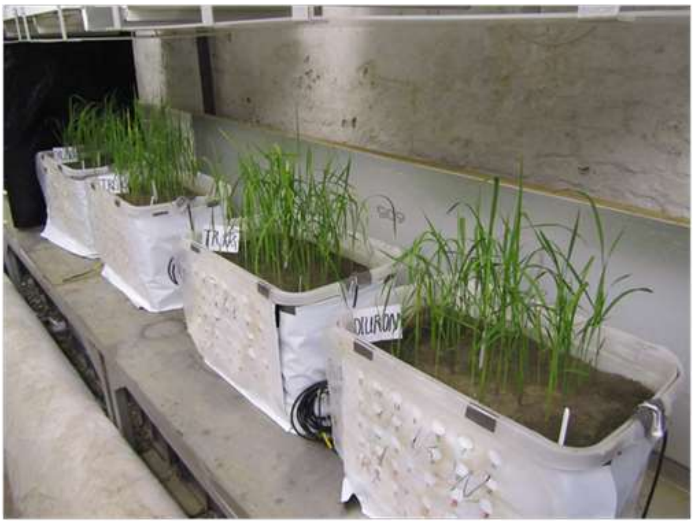

Figure 5: A total of 17 different rice genotypes where growing in four plastic boxes.

3 Statistical analysis:

Statistical analysis was conducted using Minitab 15 (State College, PA). A 5% level of significance was chosen. best subsets regression was used to reveal which combination of mean traits from each of the different root screening methods best explained the variation in herbicide score.

4 Results and Discussion:

4.1 The effect of buried TRIK herbicide on 32 different rice cultivars *(Oryza sativa L.*

Throughout the experiment, soil water content was measured regularly using a theta probe and the means of theta probe readings are presented in Figure 6. As plants grew, observations were made to determine whether symptoms of TRIK herbicide phytotoxicity were present. By about day 21 it was evident that symptoms were developing on some cultivars and data were formerly recorded from that point. On average symptoms developed progressively and in a linear fashion until about day 45 when a number of cultivars had scores of 5 (were dead) and the rate of increase therefore slowed (Figure 7). Evidence of herbicide phytotoxicity on leaves of 32 different rice cultivars started at different dates, and was significantly different on most days indicating that the 32 cultivars differed in their access to TRIK or in sensitivity to TRIK herbicide. Two-way ANOVA with factors genotype and block revealed no significant block effect ($P > 0.05$). The results of the herbicide symptoms score on days 21, 35 and 68 are presented in Figures 8 to 10. One-way ANOVA of herbicide score in cultivars on different dates revealed that the F value and R^2 increased in significance from day 21 until day 35 while the significance slowly decreased from that date as a greater proportion of varieties reached maximum scores (Figure 11) indicating that the score on 35 days was the most discriminatory between cultivars.

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The results indicated that Black Gora, N 22, Kinandang Patong, PYF3-26-5-18, Aswina, Dular, Rayda and Azucena had high scores while IR 64-21, Akihikari and M202 had low scores and Swarna and Tainung 67 did not show any symptoms even by the end of the experiment. Plants without symptoms were extracted (with the roots intact) from the boxes and maximum root length was measured. This revealed that Swarna and Tainung 67 cultivars had short roots (length ≤ 29 cm). The same 32 rice cultivars were assessed in a rhizotron experiment in the summer of 2009 (using methodology described in (13), a separate expermint using non-woven fabric in summer 2009 described in (1) and in hydroponics in summer 2010 (using methodology described in (12). Both the herbicide score and the plant height measured here correlated strongly with some traits measured in the rhizotron (Table 1), hydroponics (Table 2) and non-woven fabric (Table 3) experiments. It is noteworthy that there is high likelihood of Type 1 errors that justifies the caution over the interpretation of these marginally significant results reported in Table 1-3. To reduce the risk of this type of error, setting a higher level, such as 99%, is a way to give confidence over the significance of correlation. A best subsets regression showed that herbicide score at day 35 was most strongly correlated to seven traits of the rhizotron experiment. The strongest single trait was the number of roots passed 50 cm which alone explains 49.5 % of variation in herbicide scores (Table 4). Then, the best model with two traits is number of roots past 50 cm and root angle at 21 days which explains 54.9% of variation. With three traits, which number are of roots past 50 cm and root angle at 21 days and root thickness explains 61.1% of variation in herbicide score. A total of 67% of variation is explained by four traits root angle at 21 days, root thickness (mm), water used in three days (ml) and number of roots passed 25 cm at 21 days. All the traits above with % root mass and water used in one day (ml) explain 74.1% of variation (Table 4). No single trait in the hydroponics or non-woven experiments explained more variation than 43.3% and 37.6% respectively in herbicide score (Table 5 and Table 6). The result indicates that a much higher proportion of variation in herbicide score was explained by traits in the rhizotronexperiment.

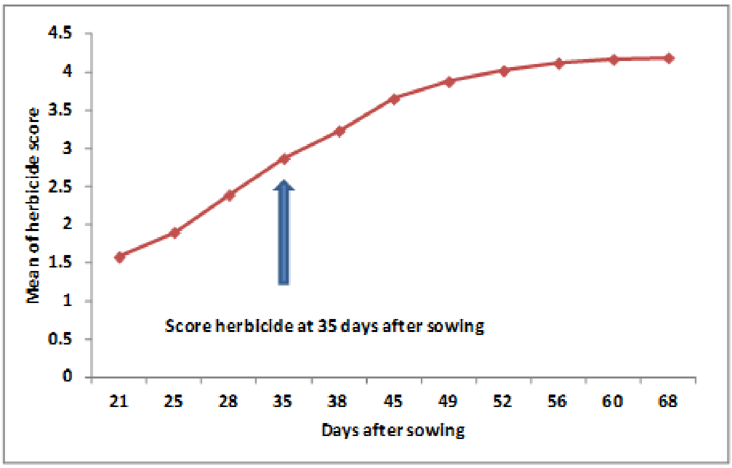

Figure 6: Mean of theta probe readings during experiment.

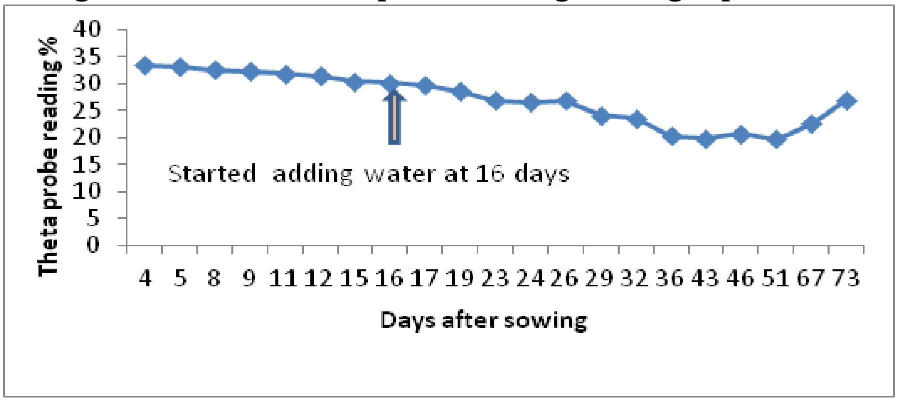

Figure 7: Mean of TRIK herbicide score at different days.

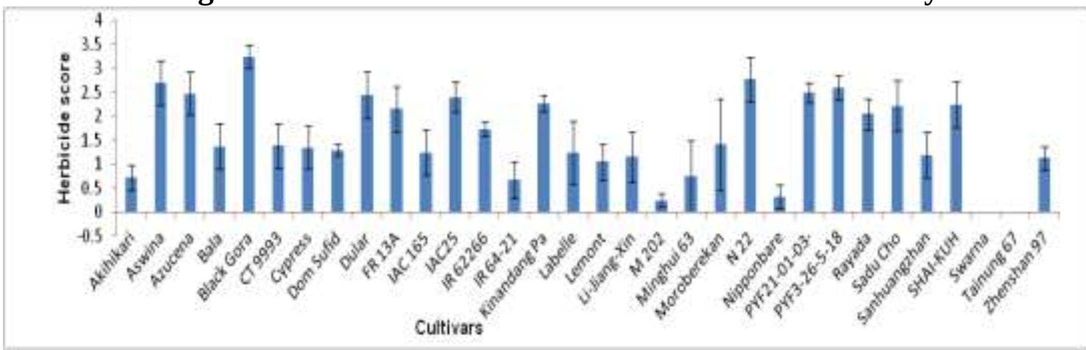

Figure 8: Score plant's leaves affected by TRIK herbicide 21 days after sowing with standard error bars.

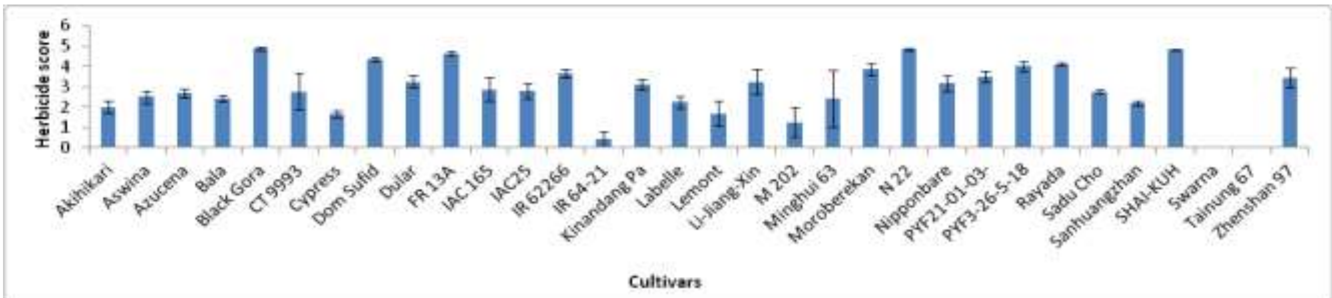

Figure 9: Score plant's leaves affected by TRIK herbicide 35 days after application with standard error bars.

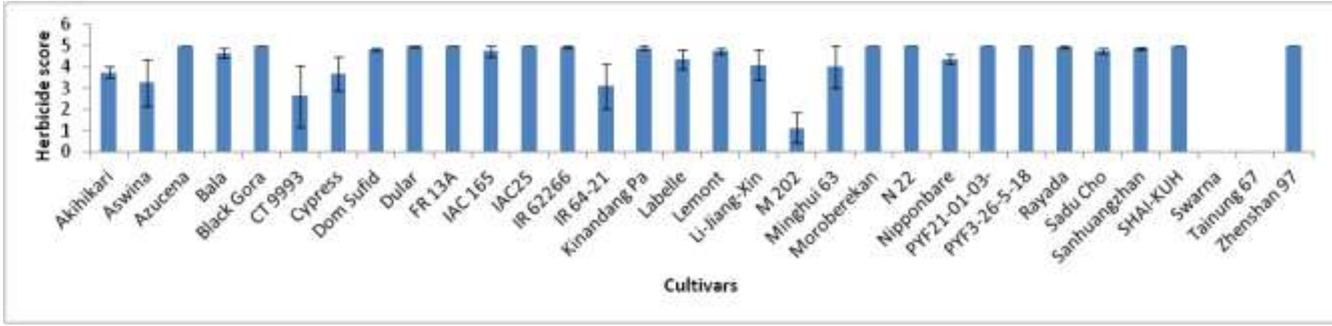

Figure 10: Score plant's leaves affected by TRIK herbicide 68 days after application with standard error bars

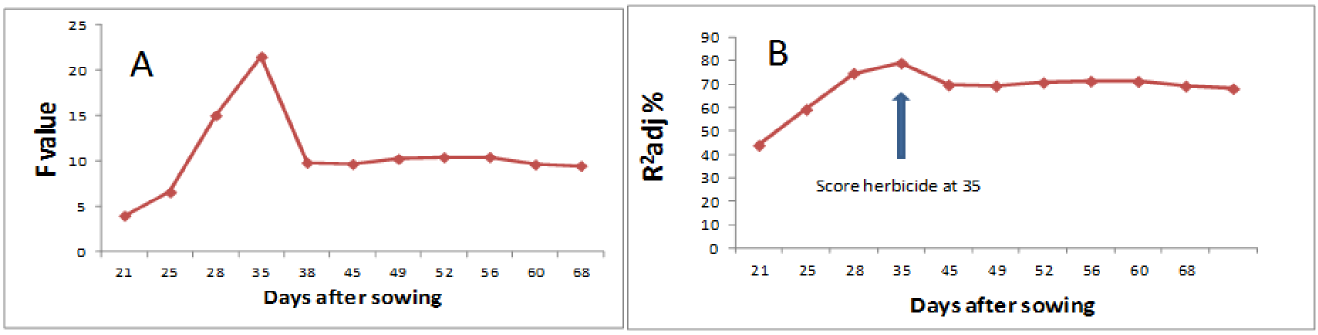

Figure 11: (A); F value and (B); R^2adj of score leaf area affected by herbicide at different days

Table1: Correlation coefficients between selected traits of herbicide score and rhizotron traits of potential influence on plant growth. n =4

		Herbicide score at different days after sowing											Plant height (cm)		
		21	25	28	35	38	45	49	52	56	60	68	7 DAS	14 DAS	21 DAS
Selected traits from rhizzotron experiment	Total plant mass (g)	0.291ns	0.476**	0.506**	0.568**	0.599**	0.540**	0.478**	0.475**	0.455*	0.456*	0.458*	0.179ns	0.221ns	0.234ns
	%Root mass	0.099ns	0.095ns	0.306ns	0.411*	0.485**	0.504**	0.436*	0.406*	0.379	0.355*	0.340ns	-0.225	-0.118	-0.083
	% of roots in bottom third	0.559**	0.529**	0.580**	0.514**	0.473*	0.428*	0.369*	0.357*	0.331ns	0.314ns	0.308ns	0.286ns	0.469*	0.371*
	Mass of root in bottom third (g)	0.524**	0.541**	0.581**	0.537**	0.529**	0.468*	0.391*	0.379*	0.349ns	0.331ns	0.324ns	0.271ns	0.393*	0.332ns
	Water used in 3 days (l)	0.453*	0.546**	0.541**	0.585**	0.603**	0.555**	0.484ns	0.463*	0.439*	0.434*	0.433*	0.291ns	0.385*	0.365*
	Root length day 21 (cm)	0.517**	0.439*	0.526**	0.498**	0.490**	0.480**	0.403*	0.369*	0.352ns	0.341ns	0.337ns	0.336ns	0.503**	0.512**
	Root length day 28 (cm)	0.595**	0.545**	0.647***	0.625***	0.583**	0.563**	0.507**	0.488**	0.472*	0.470*	0.467*	0.347ns	0.469*	0.424*
	Root length day 35 (cm)	0.491**	0.465*	0.593**	0.624***	0.604**	0.580**	0.511**	0.482**	0.461*	0.446*	0.439*	0.244ns	0.380*	0.332ns
	Root length day 42 (cm)	0.435*	0.381*	0.513**	0.544**	0.527**	0.518**	0.450*	0.423*	0.402*	0.392*	0.386*	0.218ns	0.381*	0.318ns
	No of roots passed 25 at day 21	0.296ns	0.373*	0.567**	0.594**	0.567**	0.528**	0.462*	0.429*	0.408*	0.385*	0.379*	0.132*	0.195ns	0.274ns
	No of roots passed 50 at day 35	0.463*	0.516**	0.704***	0.704***	0.656***	0.612***	0.562**	0.533**	0.516**	0.499**	0.494ns	0.136ns	0.247ns	0.260ns
	No of roots passed 75 at day 42	0.539**	0.602**	0.712***	0.670***	0.605**	0.565**	0.524**	0.503**	0.491**	0.483**	0.478**	0.163ns	0.290ns	0.297ns
	Shoot length day 21 (cm)	0.659***	0.737***	0.688***	0.635***	0.641***	0.583**	0.538**	0.510**	0.481**	0.479**	0.478**	0.571**	0.817***	0.834***
	Shoot length day 28 (cm)	0.410*	0.506**	0.530**	0.542**	0.516**	0.487**	0.428*	0.388*	0.385*	0.366*	0.365*	0.456*	0.735***	0.785***

*, **, *** Significant at *P* = 0.05, *P* = 0.01 and *P* = 0.001 respectively; ns, not significant.

Table 2: Correlation coefficients between selected traits of herbicide score and hydroponic traits of potential influence on plant growth. n =4

		Herbicide score at different days after sowing											
	Parameters	**21**	**25**	**28**	**35**	**38**	**45**	**49**	**52**	**56**	**60**	**68**	**Shoot length day 21**
Selected traits of hydroponic experiment	**Root length 7 DAS**	0.223ns	0.276ns	0.385*	0.479*	0.437*	0.47*	0.464*	0.444*	0.415*	0.398*	0.391*	0.097ns
	Root length 14 DAS	0.327ns	0.41*	0.425*	0.511**	0.469*	0.508*	0.523**	0.496*	0.466*	0.447*	0.437*	0.215
	Root length 21 DAS	0.336ns	0.313ns	0.277ns	0.315n	0.321ns	0.419*	0.446*	0.441*	0.416*	0.416*	0.405*	0.143ns
	Root length 28 DAS	0.351ns	0.335ns	0.302ns	0.353ns	0.348ns	0.44*	0.447*	0.438*	0.409*	0.406*	0.395*	0.21ns
	Root length 35 DAS	0.48*	0.418*	0.381ns	0.454*	0.445*	0.527**	0.503*	0.484*	0.448*	0.436*	0.425*	0.299ns
	Root dry weight	0.294ns	0.254ns	0.201ns	0.353ns	0.377ns	0.466*	0.495*	0.468*	0.445*	0.107ns	0.102ns	0.314ns
	% Root mass	0.226ns	0.055ns	0.191ns	0.175ns	0.178ns	0.211ns	0.169ns	0.132ns	0.127ns	0.366ns	0.357ns	0.141ns
	Root thickness	0.316ns	0.199ns	0.219ns	0.351ns	0.353ns	0.459*	0.445*	0.413*	0.39*	0.265ns	0.254ns	0.355ns
	Shoot length 7 DAS	0.167ns	0.299ns	0.156ns	0.286ns	0.26ns	0.249ns	0.311ns	0.292ns	0.272ns	0.324ns	0.313ns	0.292ns
	Shoot length 14 DAS	0.23ns	0.299ns	0.199ns	0.362ns	0.412*	0.425*	0.43*	0.385*	0.355ns	0.371ns	0.36ns	0.5*
	Shoot length 21 DAS	0.193ns	0.31ns	0.251ns	0.406*	0.396*	0.405*	0.44*	0.42*	0.391*	0.455*	0.445*	0.396*
	Shoot length 28 DAS	0.178ns	0.276ns	0.26ns	0.419*	0.431*	0.462*	0.513**	0.505*	0.48*	0.435*	0.425*	0.361ns
	Shoot length 35 DAS	0.192ns	0.331ns	0.294ns	0.43*	0.418*	0.437*	0.493*	0.48*	0.458*	0.426*	0.413*	0.417*
	Shoot dry weight	0.254ns	0.254ns	0.177ns	0.332ns	0.359ns	0.435*	0.478*	0.464*	0.443*	0.427*	0.414*	0.309ns
	Plant mass	0.265ns	0.259ns	0.186ns	0.338ns	0.363ns	0.442*	0.482*	0.466*	0.444*	0.06ns	0.056ns	0.312ns
	Root/Shoot ratio	0.241ns	0.12ns	0.242ns	0.156ns	0.14ns	0.15ns	0.107ns	0.076ns	0.077ns	0.06ns	0.056ns	0.148ns

***, **, *** Significant at *P* = 0.05, *P* = 0.01 and *P* = 0.001 respectively; ns, not significant.**

Table 3: Correlation coefficients between selected traits of herbicide and non-woven fabric traits of potential influence on plant growth. n =4.

		Herbicide score at different days after sowing							
	Parameters	**21**	**25**	**28**	**35**	**38**	**45**	**60**	**68**
Traits from non-woven fabric	**Score root penetrated at 17 DAS**	0.306ns	0.402*	0.41*	0.47*	0.4*	0.40*	0.39*	0.37ns
	Score root penetrated at 24 DAS	0.35ns	0.44*	0.43*	0.45*	0.44*	0.39*	0.39*	0.378ns
	Score root penetrated at 31 DAS	0.404*	0.491*	0.43*	0.471*	0.464*	0.424*	0.426*	0.414*
	Score root penetrated at 38 DAS	0.381ns	0.462*	0.403*	0.453*	0.445*	0.414*	0.411*	0.4*
	Plant Mass(g)	0.327ns	0.527**	0.452*	0.418*	0.404*	0.376ns	0.372ns	0.367ns
	Root Mass (g)	0.293ns	0.434*	0.399*	0.421*	0.404*	0.373ns	0.367ns	0.361ns
	Mass of root penetrated	0.315ns	0.442*	0.476*	0.449*	0.435*	0.406*	0.403*	0.396*
	%Root mass emerged	0.376ns	0.441*	0.4*	0.313ns	0.318ns	0.299ns	0.306ns	0.305ns
	Plant height at 17 DAS (cm)	0.389ns	0.554**	0.407*	0.195ns	0.22ns	0.207ns	0.219ns	0.229ns
	Plant height at 24 DAS (cm)	0.424*	0.56**	0.4*	0.26ns	0.285ns	0.272ns	0.284ns	0.291ns
	Plant height at 31 DAS (cm)	0.414*	0.475*	0.325ns	0.29ns	0.31ns	0.304ns	0.306ns	0.313ns
	Plant height at 38 DAS (cm)	0.457*	0.484*	0.383ns	0.307ns	0.32ns	0.313	0.307	0.313

*, **, *** Significant at *P* = 0.05, *P* = 0.01 and *P* = 0.001 respectively; ns, not significant.

Table 4: Best subset regression for herbicide score at 35 days after sowing with some rhizotron traits.

Variation explained %	**No .of roots passed 50 cm at 35 days**	**Root angle at day 21**	**Root thickness (mm)**	**Water used in third day (ml)**	**No .of roots passed 25 cm at 21days**	**% Root mass**	**Water used in one day (ml)**
49.5	X						
54.9	X	X					
61.1	X	X	X				
67.7		X	X	X	X		
71.9		X	X	X	X	X	
74.1		X	X	X	X	X	X

Table 5: Best subset regression for herbicide score at 35 days after sowing with some hydroponic traits

Variation explained %	Root length at day14 (cm)	Root length at day21(cm)	Root length at day28(cm)	Root length at day 35(cm)	% Root mass	Root dry weight (g)	Root thickness(mm)
26.2	X						
34.5	X	X					
40.4	X		X	X			
41.9	X	X	X	X			
42.5	X	X	X	X	X		
43.3	X	X	X	X	X	X	X

Table 6: Best subset regression for herbicide score at 35 days after sowing with some non-woven fabric traits

Variation explained %	Root penetrated score at day 17	Root mass penetrated (g)	% Root mass penetrated	Root penetrated score at day 24	Root penetrated score at day 31	Root penetrated score at day 38
27.1	X					
31.2		X	X			
34.5	X	X	X			
35.2	X	X	X			
36	X	X	X	X	X	
36.8	X	X	X	X	X	
37.2	X	X	X	X	X	X

4.2 Comparing TRIK and Diuron herbicides

Throughout the experiment for both TRIK and Diuron treatments, herbicide symptoms first became apparent on day 14 and continued to get stronger until the experiment was stopped at 35 days. Two way ANOVA with factors treatment and block revealed that there was no significant block or treatment effect on herbicide score from day 14 to 35 days after sowing (DAS).

The mean herbicide scores (across all 17 cultivars) are represented in Figure 12, which shows that there was a very similar response to both herbicides. From a regression (Figure 13) it is clear that there was a strong relationship between the score for TRIK and Diuron when examined at day 35 showing that Black Gora and Dom Sufid had high scores while IR 64-21, Zhenshan and Bala were the lowest for both herbicides. Both the TRIK and Diuron herbicide scores correlated strongly from day 14 to day 35 ($0.899 > r > 0.896$). Two way (ANOVA) with responses score showed that there was significant variation between genotypes but not for either treatments or genotype * treatment interaction (Table 7). The same 13 rice cultivars have been evaluated in a rhizotron experiment. Regression of mean herbicide scores for Diuron herbicide at 35 DAS against root length at the same date and water use at 3 DAS (from the rhizotron experiment) revealed that there were strong relationships among these traits: Black Gora had the highest herbicide score (4), root length (106 cm) and water use at 3 DAS (0.33 ml) (Figure 14). By comparison, IR 64-21 had the lowest values for these traits (0.25, 63 cm and 0.24 ml respectively).

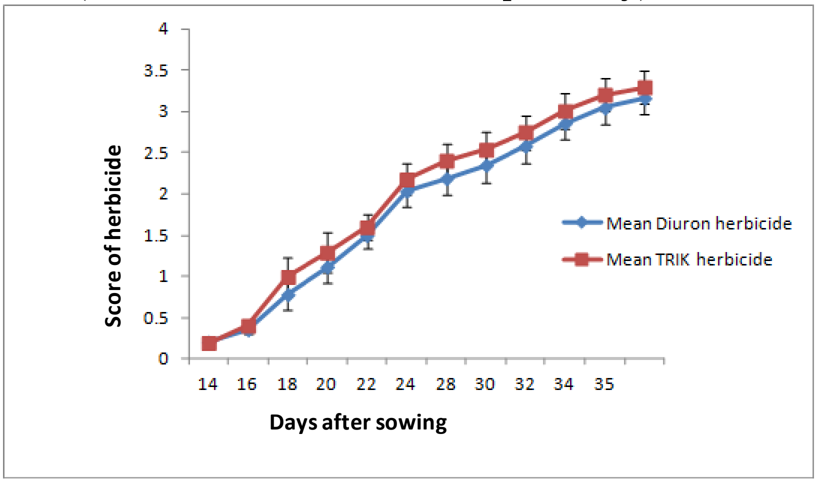

Figure 12: Mean of both herbicide score TRIK and Diuron with different dates.

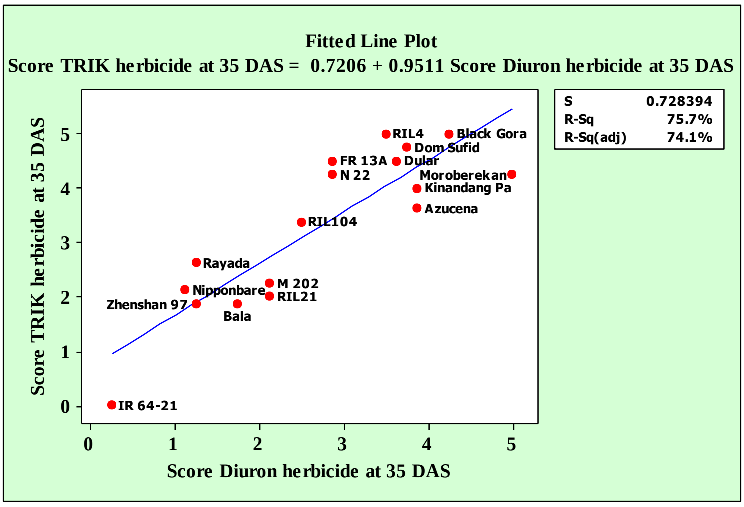

Figure13: Mean of score TRIK against the mean of score Diuron herbicide at 35 DAS.

Table 7: Two-way ANOVA were generated from score herbicide at different days

Date of Herbicide score	Varieties F	Varieties *P*	treatment F	treatment *P*	varieties*treatment F	varieties*treatment *P*
14 DAS	8.19	<0.001	0.34	0.5	0.5	0.9
16 DAS	6.89	<0.001	2.96	0.08	0.75	0.74
18 DAS	2.94	<0.001	3.4	0.06	1.2	0.28
20 DAS	3.29	<0.001	2.3	0.13	1.17	0.30
22 DAS	7.01	<0.001	1.65	0.2	1.56	0.09
24 DAS	6.34	<0.001	2.17	0.14	1.62	0.07
28 DAS	6.32	<0.001	2.95	0.08	1.39	0.16
30 DAS	8.53	<0.001	2.71	0.1	1.00	0.40
32 DAS	12.5	<0.001	2.85	0.09	0.89	0.57
34 DAS	12.85	<0.001	3.42	0.06	0.89	0.58
35 DAS	15.48	<0.001	2.52	0.11	0.91	0.55

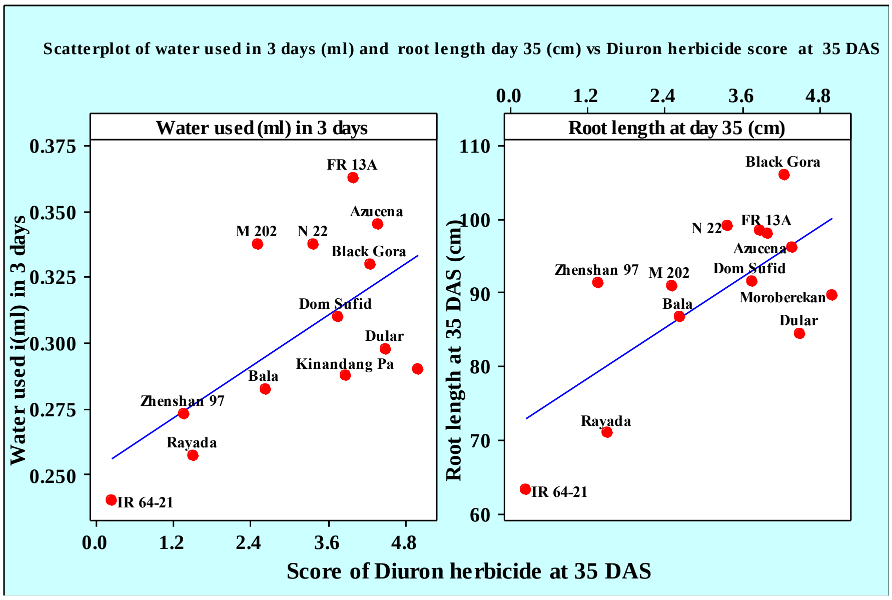

Figure 14: Mean of Diuron herbicide score at 35 DAS against the mean of water use (ml) at 3 DAS (Left) and root length (cm) at 35 DAS (Right) (Data from rhizotron experiment).

In this bioassay study, the score of leaf area affected by herbicide was used as an indicator of rooting depth based on an assumption that when the roots reach the herbicide, they take it up and the leaves will die. The approach assumes that the mobility of the herbicide towards the surface is limited, which is confirmed by (11) who showed that capillary rise of water from a subsurface could be minimal in several soils. In the current study of 32 different rice cultivars, substantial variation in herbicide symptoms between cultivars was detected. This was most apparent at 35 days after application. By the end of the experiment, most plants were dead, yet some were not. It is assumed that the speed with which symptoms appear reflect how rapidly roots get down the 30 cm to the herbicide layer. Digging up the roots indicated that the vast majority of those plants without symptoms had root systems that did not reach the herbicide layer. However, M202 roots were deep enough to contact the herbicide yet it had few symptoms. This suggests that this cultivar has a lower sensitivity to the herbicide. (14) noted that the screening approach would not be capable to observe varieties that have resistance to herbicide and deep rooting, but suggested that this was not important since genotypes of this combination are probably very rare. In addition, it can be argued that major differences in sensitivity, such as the apparent resistance of M202, might be accommodated in a screening programme if they are rare if the main aim is to identify deep rooting individuals from a large collection such as a mutant screen, where the interesting genotypes are those with rapid symptoms (and therefore deep roots).

The herbicide technique has previously permitted determination of variation in rooting as a function of origin, genotype and soil water content. Corre-Hellou and Crozat (2005) noted that the herbicide technique where herbicide is injected into field soil is destructive but permits a large number in situ repetition and does not disturb the soil profile. However, is important to note that the reaction time was quicker during sunny than cloudy weather. This difference in response time would be predicted for a compound that must be translocated to the shoots, presumably through the transpiration stream. Similarly, hotter days may speed up symptom development as would sun if the herbicide's mode of action was via interaction with photosynthesis and the higher temperatures may improve root growth rate (5).

There were strong correlations between herbicide score reported here and root traits measured with three techniques, especially the rhizotrons. These correlations strongly imply that symptoms are related to root development and transpiration demand (shoot mass or water use) and are therefore ideal for a screen of access to water at depth. The Oryza SNP set of genotypes is being widely examined for traits linked to roots in field experiments. So far 19 Oryza SNP genotypes have been tested in field experiments for 3 seasons to estimate yield in irrigated and aerobic fields and measure root density at 15 cm interval depths (6). However more studies need to be done in order to harness data above to set up future experiments.

Unfortunately TRIK herbicide which was used in our study has been banned globally and cannot now be purchased. We decided to explore the use of Diuron for two reasons. Firstly, it is one of the active ingredients of TRIK and indeed TRIK is almost 46.7% Diuron. Secondly, Diuron is a readily available research chemical so future researchers will not have difficulty obtaining it.

A total of 17 genotypes were selected to test their responses to both TRIK and Diuron herbicide. The selection depended on their score at 35 DAS in the previous experiment.There was a very substantial level of agreement between the two treatments both in terms of the speed with which the mean score developed and in the correlation between cultivars at particular time points in that development. This result encourages us to apply Diuron herbicide in future experiments. It is worth noting that M202 was included in this experiment. In the previous experiment it had a very low herbicide score despite being found to have a moderately deep root in an earlier rhizotron screen (see Figure 10). It was reassuring to see that M202 had a reasonably high herbicide score, particularly with the TRIk. The logical interpeation for the low score of M202 in primary experiment is that the primary experiment was conducted in the greenhouse where day temperature was 25°C whereas the later experiment was conducted in a controlled growth room with a day temperature of 28-30°C. According to Grumet and his colleagues, the hotter condition may improve root growth rate and therefore increase herbicide symptoms rapidly.

Conclusion:

Root depth traits are known to be crucially important in the drought resistance of some rice cultivars (*Oryza sativa* L.). This experiment involves the screening of root depth by using a layer of herbicide at a depth of 30 cm in the soil as a technique for high throughput phenotyping of genotypes. This method is cost effective but it was important to establish if it could be related to known variation in root traits in the cultivars tested. Herbicide score were markedly different between rice cultivars. The comparisons between TRIK herbicide experiment and either the rhizotron, hydroponics and non-woven fabric penetration experiments showed evidence there were strong correlations which were strongest with the rhizotron experiment and weakest with the non-woven fabric experiment. A best subsets regression revealed that herbicide symptoms score from day 35 were reasonably correlated with different root traits and some shoot traits of these experiments. Most importantly it appears to relate to the amount of roots at depth and the driving force for transpiration (shoot mass or water use) which is exactly what is required for a screen of access to water at depth. It is apparent that both the TRIK and Diuron herbicides are equally effective in discriminating cultivars while Diuron should be widely available to researchers. There is variation in herbicide sensitivity between cultivars but it is not sufficiently large to compromise the value of the buried herbicide technique. It can be concluded that the herbicide technique is quick and easy to apply. However more studies need to be done to use data above with other available data to determine if the phenotype measured with these methods is relevant to performance in the field.

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