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Effect of *Trichoderma* and hydrogel on growth, yield and yield attributes of direct seeded rice (*Oryza sativa*) under rainfed condition

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ABSTRACT

A trial was conducted both in field and pot during the *kharif* season of 2015 at Banaras Hindu University, India to study the effect of hydrogel in combination with bio-agent on the performance of rice under rainfed condition. The field trial consisting of IR64 and DRR42 and *Trichoderma* and hydrogel making 10 treatments altogether was laid out in a Randomized Complete Block Design (RCBD) while in case of pot it was a factorial experiment. DRR42 + hydrogel (seed coating)+*Trichoderma* (seed treatment @ 12 g/kg) recorded higher yield (2.83 t ha^{-1}) which was 43.76% higher than control but it remained at par with IR64. In the pot experiment hydrogel (seed coating)+*Trichoderma* (seed treatment) and hydrogel soil application registered higher RGR, CGR, root length, root weight. Survival of plants after imposition of drought at 60 days after sowing (DAS) was also found to be longer under the same treatments.

Key words: Direct seeded, DRR 42, Hydrogel, IR 64, Rainfed, Seed treatment, Trichoderma.

Food security is the major challenge in India. Rice being the most important cereal crop, has a vital role to meet this challenge. More than 70% of the people of India consume rice. However, India needs to add 1.7 million tonnes of additional rice every year to ensure national food security. Among the rice growing countries in the world, India stands first in area and second in production, next only to China. In India, rice alone is cultivated on 43.86 Mha, with a total production of around 104.80 million tonnes at a productivity level of 2390 kg/ha (Agricultural Statistics at a Glance 2015).

Water is one of the most important limiting factors in crop production because it is the scarcest natural resource. Hoekstra and Chapagain (2008) estimated that India uses 2 to 4 times more water to produce one unit of major food crop than does China and Brazil. Rice is a high water demanding crop and its requirement ranges from 900 -2500 mm approximately. Out of total cultivated rice only 58.4% is under irrigation while rest is under rainfed condition.

Rainfed rice is totally dependent on precipitation where water stress at critical juncture (in case of erratic rainfall) reduces the growth and yield of rice drastically. Moisture stress can be defined as the lack of adequate moisture required for normal plant growth and development (Manivannan *et al.* 2008). By 2025, 2 M ha of Asia's irrigated dry season rice and 13 M ha of its irrigated wetland rice may suffer from physical water scarcity. Under such conditions, cell elongation inhibited by interruption of water flow from xylem to surrounding elongating cells (Nonami 1998). Growth accomplished by cell division, cell enlargement and differentiation involves genetic, physiological, ecological and morphological characters and their complex interactions. Reduced nutrient uptake and turgidity under low moisture leads to decreased growth and yield because of less photosynthesis and translocation of assimilates (Wahid and Rasul 2005).

Rice is susceptible to moisture stress where use of hydrogel improves availability of soil moisture to the plant. Hydrogels are super absorbent polymers that absorb and retain water hundreds to thousands times of their own weight, i.e. 400-1500 g water per dry gram of hydrogel (Johnson 1984). It prevents deep percolation losses and gradually releases water in the root zone at the time of stress and thus increases the amount of available moisture for crop. It acts as a reservoir of nutrients and moisture in the soil and functions like a controlled release device. Application of hydrogel at 0.7% increases the moisture at saturation by 21% in black clay soil, 40% in sandy soil and 24% in alluvial and red sandy loam soils (Rudzinski *et al.* 2002). Hydrogel also improves crop growth and development by increasing nutrient absorption of plant under limited moisture

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condition (Rahimi *et al*. 2014).

There are a number of bioagents which helps in mitigating the adverse effect of moisture stress in many crops. *Trichoderma* is one such bioagent which helps plant to recover from moisture stress. Some of the mechanisms involved in the alteration of stress response by *Trichoderma* include drought avoidance through morphological adaptations, drought tolerance through physiological and biochemical adaptations and enhanced drought recovery. It releases a variety of compounds that induce resistance to biotic and abiotic stresses (Harman *et al*. 2004). Root colonization by *Trichoderma* spp. also frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and uptake and use of nutrients. *Trichoderma* spp. colonizes roots of treated seedlings and remains restricted to the cortex and outer layers of the root epidermis (Yedidia *et al.* 2001). Several studies have shown that root colonization by *Trichoderma harzianum* results in increased level of plant enzymes, including various peroxidases, chitinases, β -1,3-glucanases, lipoxygenase-pathway hydro peroxide lyase and compounds like phytoalexins and phenols to provide durable resistance against stress (Harman 2000). It improves the seedling vigor, root and shoot length and growth of rice plant. N and P uptake of crop is reduced under moisture stress condition because of their low mobility and under such condition *Trichoderma* helps mitigate the adverse effect and improves growth and yield of crop.

MATERIALS AND METHODS

The field experiment was conducted during the *kharif* season of 2015 at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh (India). Average water requirement for rice under normal condition ranges from 900 to 1500 mm while rainfall received during the crop growth period was 543.5 mm which indicated scarcity of moisture during crop growth season. The maximum rainfall during crop growth period was 116.3 mm during 13-19 August 2015 which coincided with tillering stage (Table 1).

Table 1 Treatment details

T_1	IR 64
T_2	IR 64 (*Trichoderma*, seed treatment @ 12 g/kg)
T_3	IR 64 (hydrogel, furrow application)
T_4	IR 64 (hydrogel, seed coating +*Trichoderma* (seed treatment @ 12 g/kg)
T_5	IR 64 (*Trichoderma*, seed treatment @ 12 g/kg + hydrogel, furrow application)
T_6	DRR 42
T_7	DRR 42 (*Trichoderma*, seed treatment @ 12 g/kg)
T_8	DRR 42 (hydrogel, furrow application)
T_9	DRR 42 (hydrogel, seed coating +*Trichoderma* seed treatment @ 12 g/kg)
T_{10}	DRR 42 (*Trichoderma*, seed treatment @ 12 g/kg + hydrogel, furrow application)

The trial was conducted in a specially designed permanent plots (4 m × 4 m) having brick built 30 cm wide 180 cm deep cemented, water proof plastered wall on all sides. The main purpose of constructing these plots was to completely check the lateral movement of water from one plot to another through seepage. Each plot was fitted with separate water supplying device through pipe attached with horizontal water meter to measure the exact amount of water (litre) supplied to the plots as and when required.

The soil of the experimental field was well drained sandy clay loam with pH 7.45, EC 0.53 dS/m, organic carbon 0.37%, available nitrogen 200 kg/ha, available phosphorus 18 kg/ha and available potassium 204 kg/ha. The trial was laid out in a Randomized Complete Block Design with 3 replications. Two rice varieties IR 64 and DRR 42 were used. Hydrogel was applied in furrows and used as seed coating material as per treatment whereas *Trichoderma* was used for seed treatments (Table 1).

Both the varieties were sown directly on July 22, 2015 at a spacing of 20 cm × 20 cm by dibbling 3-4 seeds per hill. IR 64 and DRR 42 are medium early, less than 115 days duration varieties suitable for rainfed condition. While IR 64 is well established rice variety, DRR 42 is a newly developed dwarf variety in which drought resistance gene of IR 64 has been incorporated.

The same experiment was repeated in pots also with 3 replications. The trial consisting of the same two varieties along with 5 treatments making 10 treatment combinations was a factorial experiment laid out in RCBD. However, the crop was allowed to continue upto 90 days only as the observations were mostly destructive and no plant was left in the pot after the final observation was taken. Earthen pots of 5 kg capacity were selected and filled with 4 kg soil. All the pots were maintained with constant moisture upto 60 days by supplemental irrigation after which artificial moisture stress was created by complete stoppage of irrigation. In addition to that pots were put under polythene sheets also to protect them from any rainfall. CGR ($g/m^2/day$) and RGR (g/g/day) at different stages were estimated as per the methodologies of Watson (1952) and Hoffman and Poorter (2002) respectively. In each pot two hills were raised and in order to study the CGR, RGR, and root characters where destructive samplings were done (for fresh weight and dry weight at different stages) extra pots were sown with crop. For root studies the entire soil was taken out from each pot, washed gently to separate the soil from roots without any destruction of rootlets (as far as possible) and then the root length was measured by stretching it to its fullest length. For root weight, the root masses were sun dried and then put into the oven at 65ºC till the constant weight was obtained.

A uniform application of NPK @ 120-60-60 kg/ha was applied to all the plots through urea, DAP and MOP. The whole amount of P and K was applied as basal, while N was applied in 1:2:1 ratio at the time of sowing, tillering and panicle initiation stages respectively. In addition to this foliar spray of Zn @ 0.5% through $ZnSO_4$ was done at 35 days after sowing. Hydrogel was applied as basal @

2.5 kg/ha in the furrow by mixing it with the sand in 1:10 ratio. Seed treatment with *Trichoderma* and hydrogel was made as per the standard procedure and weeding was done as and when required to keep the crop weed free. Both the varieties were harvested on November 07, 2015 in the field experiment.

RESULTS AND DISCUSSION

Effect of treatments on growth

In crop growth rate (CGR) (Table 2) and relative growth rate (RGR) (Table 3) both DRR 42 and IR 64 registered equal growth at all the stages in pot experiment although a slight increase was observed in DRR 42.

However, among the seed treatments *Trichoderma* inoculation+ coating with hydrogel (T_4) and only application of hydrogel in soil (T_5) registered higher growth at all the stages of observations than other treatments, while between themselves there was no significant difference. RGR refers to the original difference in size of plants which is reflected in the production of dry mass (Hoffmann and Poorter 2002).

No significant difference in RGR indicated that leaf area ratio (LAR) and net assimilation rate (NAR), CGR and leaf area index (LAI) which influence the photosynthesis (Cho and Murata, 1980) were same for both the varieties. Contrary to this in seed treatment, *Trichoderma* and hydrogel (T_4 and T_5) were able to establish their effects on crop growth in respect of NAR , LAR and LAI and registered higher values than other treatments. Lorito *et al*. (2010) stated that the benefit of *Trichoderma* spp. in improving plant growth could be due to any of the following mechanisms like micoparasitism, degradation of toxins, resistance to pathogens and more nutrient uptake through enhanced root development. Similar trend was observed in root studies also, where varieties failed to bring about any significant difference in root length or root dry weight (Table 4) but seed treated with *Trichoderma* and hydrogel (T_4) registered maximum root length and weight although it remained at par with *Trichoderma* + hydrogel soil application (T_5).

The increase in root length and weight over control (water soaking) was found to be 119.49% and 129.68% respectively. *Trichoderma* species is known for years to increase plant growth and root biomass (Ureta *et al.* 1995*)*. In maize seedlings *T. harzianum* (T22) has been found to colonize in root and induce large changes in the shoot proteomes even though it is present only in roots. A large portion of upregulated shoot proteins increases photosynthesis resulting in increased starch accumulation in seedlings (Shoresh and Harman, 2008).

Further, survival duration after drought induction at 60 days was not found to be significantly affected by the varieties (Table 4). However, seed treated with *Trichoderma* and coated with hydrogel (T_4) significantly increased the survival duration over control but remained at par with hydrogel application in soil. The *Trichoderma* colonized rice seedlings maintained leaf greenness for longer time and were slower to wilt because of reduced stomatal conductance. (Shukla *et al*. 2012).

Table 2 Crop Growth Rate (CGR) (g/m²/day) as affected by treatments

Treatment	0-30 DAS	30-60 DAS	60-90 DAS
IR 64	0.128	1.554	4.934
DRR 42	0.140	1.793	5.169
SEm±	0.002	0.032	0.031
CD (P = 0.01)	NS	NS	NS
T1	0.061	0.958	4.092
T2	0.097	1.199	4.593
T3	0.124	1.672	5.200
T4	0.160	2.043	5.598
T5	0.153	1.995	5.523
SEm±	0.004	0.051	0.049
CD (P = 0.01)	0.017	0.211	0.202

T1, Water soaked seed; T2 = Seed treatment with *Trichoderma*; T3 = Hydrogel applied in soil; T4 = Seed treatment with *Trichoderma* +Hydrogel; T5 = Seed treatment with *Trichoderma* +Hydrogel applied in soil.

Table 3 Relative Growth Rate (RGR) (g/g/day) as affected by treatments

Treatment	0-30 DAS	30-60 DAS	60-90 DAS
IR 64	0.052	0.635	2.097
DRR 42	0.060	0.717	2.183
SEm±	0.001	0.012	0.013
CD (P = 0.01)	NS	NS	NS
T1	0.036	0.374	1.735
T2	0.042	0.460	1.934
T3	0.054	0.663	2.195
T4	0.069	0.829	2.374
T5	0.066	0.804	2.336
SEm±	0.001	0.051	0.022
CD (P = 0.01)	0.004	0.211	0.091

Table 4 Effect of treatments on root dry weight wt (g), root length (cm) at 70 DAS and survival duration after induction (days)

Treatment	Length	Weight	Days
IR 64	46.06	9.13	4.03
DRR 42	56.46	11.05	4.36
SEm±	0.60	0.22	0.064
CD (P = 0.01)	2.44	0.90	0.26
T1	30.83	5.66	4.0
T2	38.50	8.33	4.0
T3	53.00	11.00	4.80
T4	67.67	13.00	4.83
T5	66.33	12.33	4.16
SEm±	0.95	0.34	0.102
CD (P = 0.01)	3.92	1.40	0.421

Effect of treatments on yield attributes of rice

It was clear from the weather data that from September onwards there was hardly any precipitation and with temperature remaining high it had to pass almost entire reproductive stage under moisture stress condition. Plenty of literature is available to state that during reproductive stage plant need adequate moisture for anthesis, grain formation and filling etc. (Saini and Westgate 1999 and Sarvestani *et al.* 2008).

Data related to yield attributes clearly revealed that seeds of DRR 42 when coated with hydrogel and treated with *Trichoderma* @ 12 g/ kg seed produced higher number of longer and heavier panicles/m^2 and grains/ panicle than all others treatments (Table 5). The increase in yield attributes might be due to secretion of growth promoting substances by *Trichoderma* in host plant which helped plant perform better under moisture stress condition. Mathivanan *et al.* (2006) reported that *Trichoderma* increased the level of scavenging enzymes like super oxide dismutase (SOD), peroxidase, glutathione-reductase and glutathione-s-transferase (GST) in the leaves of treated plant which detoxify the super oxides and free radicles generated during stress in plant. It triggers a series of mechanisms in host plant which enhance natural defenses against biotic and abiotic stresses (Shukla *et al.* 2012). Besides this, it has the ability to produce siderophores, phosphate-solubilizing enzymes, and phytohormones. Plant roots colonized by microbes under moisture stress conditions can reduce stomatal conductance and maintain leaf water potential (Augé 2001). It was further observed that relationship between yield and other yield parameters as influenced by the treatments was same in both the varieties (Fig 1 - Fig 4) except panicle length and grain yield where the relationship was found to be quadratic in case of DRR 42 most probably because of higher number of unfilled spikelets in this variety.

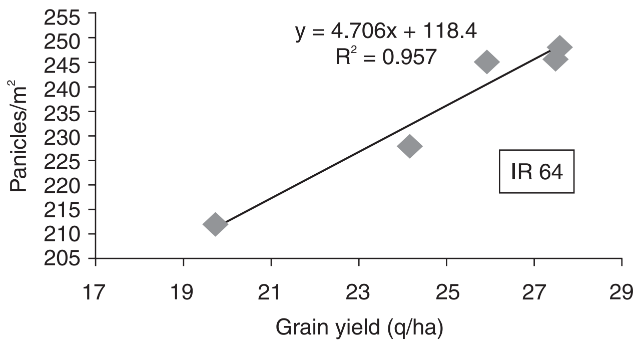

Fig 3 Functional relationship between panicle per m^2 and grain yield.

Cumulative effects of all these parameters ultimately led to similar grain yield by the DRR 42 and IR 64 which remained statistically at par with each other. Hydrogel (seed coating) + *Trichoderma* (seed treatment @ 12 g/kg) produced maximum grain yield in DRR 42 with 2.83 t/ha which was 43.76% higher than control. This clearly indicated that hydrogel and *Trichoderma* were able to mitigate the effect of moisture stress to a great extent which was reflected in the increased yield in both DRR 42 (T_9) and IR 64 (T_4). In the correlation studies as expected both grain and straw yields were found to be positively and significantly

Table 5 Effects of treatments on yield and yield attributes of rice

Treatment	No. of panicles/ m^2	Panicle length (cm)	Panicle weight (g)	No. of grains / panicle	Test weight (g)	Grain yield (q/ha)	Straw yield (q/ha)	Harvest index (%)
IR 64	212.00	20.33	2.60	70.37	20.50	19.71	26.55	42.77
IR 64 (T*richoderma*, seed treatment @ 12 g/kg)	227.83	21.10	2.76	76.03	21.07	24.07	32.96	42.14
IR 64 (hydrogel, furrow application)	245.17	20.80	2.95	81.27	20.87	25.89	34.32	44.42
IR 64 (hydrogel, seed coating + *Trichoderma*, seed treatment @ 12 g/kg)	248.33	21.80	3.02	81.30	20.59	27.59	37.04	42.68
IR 64 (*Trichoderma*, seed treatment @ 12g/ kg + hydrogel, furrow application)	246.00	21.60	2.96	80.20	20.15	27.44	35.40	42.40
DRR 42	224.67	20.10	2.73	70.67	22.33	20.48	27.23	42.82
DRR 42 (*Trichoderma*, seed treatment @ 12 g/kg)	228.33	20.87	2.85	78.73	20.00	23.11	33.00	41.17
DRR 42 (hydrogel, furrow application)	243.67	21.37	2.94	80.70	23.31	26.29	36.37	41.92
DRR 42 (hydrogel , seed coating + *Trichoderma*, seed treatment @ 12 g/kg)	255.00	22.27	3.06	82.37	22.25	28.32	38.74	42.30
DRR 42 (*Trichoderma*, seed treatment @ 12 g/kg + hydrogel, furrow application)	229.33	21.43	2.80	75.70	21.41	22.44	30.68	42.24
SEm±	5.01	0.31	0.09	1.42	0.68	0.94	1.30	1.13
CD (P=0.05)	14.88	0.92	0.27	4.22	NS	2.79	3.86	NS

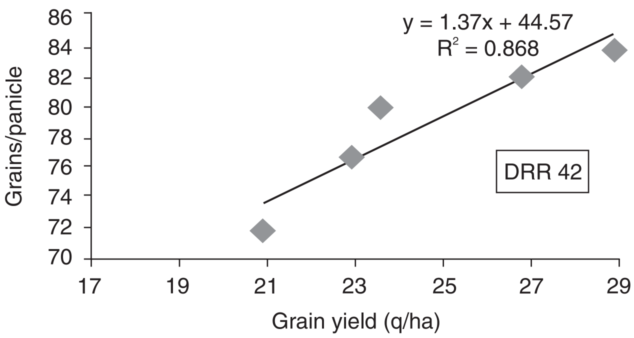

Fig 2 Functional relationship between grains/panicle and grain yield.

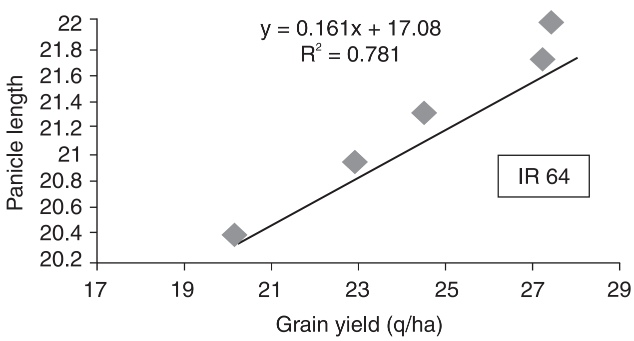

Fig 3 Functional relationship between panicle length (cm) and grain yield.

correlated with panicle number, length, weight and number of grains/panicle (Table 6).

Effect of treatments on nutrient uptake by rice

It is well known that the uptake of nutrient is reduced under moisture stress condition. The reduction might be due to reduced transpiration (O'Toole and Baldia 1982) and/or impaired transport of nutrient. A decline in soil moisture resulted in decrease diffusion rate of nutrients from the soil matrix to the absorbing root surface (Marais and Weirsma 1975), thereby reducing their uptake.

The data pertaining to N, P and K uptake clearly showed that maximum amount of N, P and K was taken up by DRR 42 under hydrogel (seed coating) + *Trichoderma* (seed inoculant @ 12 g/kg) treatment but it remained at par with IR 64 under the same treatment (Table 7).

Table 6 Correlation coefficient of the yield and yield attributes

	Panicles/m^2	Panicle length	Panicle weight	Grains/panicle	Test weight (g)	Grain yield	Straw yield
Panicle length	0.771*						
Panicle weight	0.970**	0.698					
Grains/Panicle	0.919**	0.639	0.952**				
Test weight	0.215	0.107	0.120	0.082			
Grain yield	0.959**	0.746*	0.948**	0.968**	0.121		
Straw yield	0.930**	0.833*	0.941**	0.941**	0.131	0.959**	
Harvest Index	0.152	-0.244	0.072	0.144	-0.038	0.185	-0.099

**Significant at 1% , *Significant at 5%

Table 7 Effects of treatments on nutrient uptake by rice

Treatment	N uptake (kg/ha)		P uptake (kg/ha)		K uptake (kg/ha)	
	Grain	Straw	Grain	Straw	Grain	Straw
IR 64	24.05	19.12	4.53	2.12	6.11	46.47
IR 64 (T*richoderma*,seed treatment @ 12 g/kg)	29.37	23.40	5.54	2.27	7.46	65.93
IR 64 (hydrogel ,furrow application)	33.48	25.05	6.31	2.78	8.51	64.86
IR 64 (hydrogel,seed coating+*Trichoderma* ,seed treatment @ 12 g/kg)	33.66	27.41	6.35	3.04	8.55	73.71
IR 64 (*Trichoderma* , seed treatment @ 12 g/kg + hydrogel, furrow application)	31.58	25.49	5.95	2.76	8.02	63.02
DRR 42	24.98	19.88	4.71	2.26	6.35	48.47
DRR 42 (*Trichoderma* , seed treatment @ 12 g/kg)	28.19	22.77	5.31	2.61	7.16	61.05
DRR 42 (hydrogel, furrow application)	32.08	25.46	6.05	2.98	8.15	72.74
DRR 42 (hydrogel , seed coating + *Trichoderma*, seed treatment @ 12 g/kg)	34.55	28.28	6.51	3.22	8.78	75.54
DRR 42 (*Trichoderma*, seed treatment @ 12 g/kg + hydrogel, furrow application)	27.38	21.78	5.16	2.49	6.96	55.22
SEm±	2.09	0.98	0.21	0.11	0.49	2.41
CD(P=0.05)	6.04	2.90	0.64	0.32	1.47	7.32

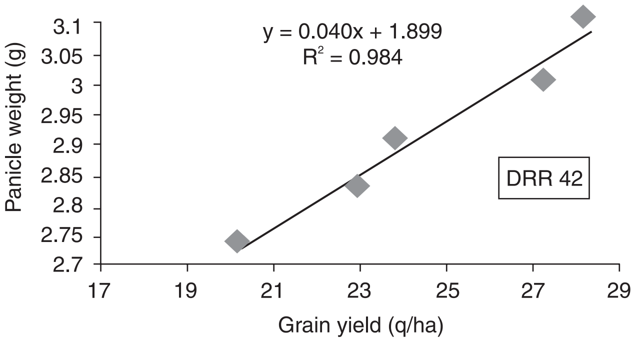

Fig 4 Functional relationship between panicle weight (g) and grain yield.

This was perhaps due to more active micro-organism along with enhanced root activity which led to more solubilisation of nutrients around the rhizosphere of rice facilitating higher uptake of nutrient. Application of *Trichoderma* in plants increases root depth, which help in increasing moisture acquisition, nutrient uptake and plant's ability to resist moisture stress (Arora *et al*. 1992). It is a well established fact that *Trichoderma harzianum* solubilizes insoluble or sparingly soluble minerals and help the plant take up more nutrient from soil (Saba *et al*. 2012).

It can therefore be concluded that DRR 42 or IR 64 when sown after being coated with hydrogel and inoculated with *Trichoderma* can perform better under moisture stress condition.

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