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V Sumanth
Department of Genetics and Plant Breeding, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences Prayagraj, Uttar Pradesh, India

G Roopa Lavanya
Department of Genetics and Plant Breeding, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences Prayagraj, Uttar Pradesh, India

Corresponding Author:
V Sumanth
Department of Genetics and Plant Breeding, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences Prayagraj, Uttar Pradesh, India

Studies on direct and indirect effects at seedling stage stress conditions in early backcross population of rice (*Oryza sativa* L.)

V Sumanth and G Roopa Lavanya

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Abstract
Rice (*Oryza sativa* L.) stands as one of the most crucial staple crops worldwide providing sustenance for over half of the global population. This study evaluated a total of 155 BC1F3population were screened under sand culture at seedling stage and reproductive stage with tolerant check Dular as positive control and improved lalat as susceptible at Crop Improvement Division, ICAR-National Rice Research Institute-Cuttack, focusing on yield and grain yield related traits under nutrient stress conditions. Significant genetic variability was observed for traits such as SPAD, shoot length and root length. Strong direct effect of root length on root dry weight in both genotypic and phenotypic path analysis echoes findings that taller plants often allocate more resources to root growth.

Keywords: Rice, *Oryza sativa*, variability, genotypic and phenotypic path analysis

1. Introduction
Rice, scientifically known as *Oryza sativa* L., holds an esteemed position as one of the most indispensable staple crops on a global scale. With its ability to provide nutrition for over half of the world's population, rice serves as a fundamental source of nourishment for billions of individuals (Fanzo *et al*., 2013) [1]. The potential yield of rice is influenced by various factors, including nutrient availability in the soil. Nutrient stress, particularly deficiencies in essential elements such as nitrogen (N), phosphorus (P), and potassium (K), can significantly limit rice productivity (Dobermann and Fairhurst, 2000) [2]. In regions where soil fertility is compromised or where fertilizer application is limited due to economic constraints, nutrient stress poses a formidable challenge to rice cultivation, jeopardizing food security and livelihoods.

Direct Seeded Rice (DSR) Cultivation
Direct Seeded Rice (DSR) cultivation refers to a method of rice production where seeds are sown directly into the field without the need for nursery raising and subsequent transplantation. DSR is characterized by its simplicity, efficiency, and resource-saving nature, as it eliminates the labor-intensive process of seedling preparation and transplanting, thereby reducing costs and saving water (Pandey *et al*., 2012) [3]. In DSR, seeds are typically broadcast or drilled into well-prepared fields with adequate soil moisture, facilitating rapid germination and establishment of seedlings (Fischer *et al*., 2019) [4]. This method offers flexibility in planting time, enabling farmers to synchronize planting with favorable weather conditions and optimize crop establishment. Direct Seeded Rice (DSR) has gained attention as a viable alternative to traditional transplantation methods due to its numerous benefits, including significant water conservation, reduced labor requirements, earlier crop maturity, and lower greenhouse gas emissions. DSR can decrease water use by up to 50%, making it an attractive option in water-scarce regions (Farooq *et al*., 2011) [5]. It also eliminates the labor-intensive process of transplanting seedlings, thereby cutting labor costs and increasing efficiency (Kumar and Ladha, 2011) [6]. Furthermore, DSR can lead to earlier canopy closure and reduced weed competition, potentially resulting in higher yields (Pandey and Velasco, 2002) [19], by avoiding continuous field flooding, DSR helps reduce methane emissions, thus contributing to a lower environmental

impact (Jain *et al.*, 2019) [20]. Addressing nutrient deficiencies in DSR cultivation requires tailored management strategies to optimize nutrient availability and uptake. Soil testing and nutrient profiling are essential for diagnosing nutrient deficiencies and formulating appropriate fertilizer recommendations based on crop requirements and soil characteristics (Witt *et al.*, 2007) [8]. Furthermore, the timing and placement of fertilizer applications play a crucial role in ensuring nutrient availability during critical growth stages, particularly during the establishment phase in DSR systems (Jat *et al.*, 2019) [7].

Advantages and challenges of Sand hydroponics compared to traditional transplantation methods

Sand hydroponics, a form of soilless cultivation, offers a sustainable approach to rice farming, presenting unique advantages and challenges compared to traditional transplantation methods. One significant advantage is water conservation, as sand hydroponics allows for efficient water use through recycling and reuse within a controlled environment, minimizing wastage typical of traditional flooding methods (Senthil kumar *et al.*, 2019) [9]. Additionally, the delivery of essential nutrients directly to the plant roots in a dissolved form enhances nutrient uptake efficiency and reduces losses through leaching, contributing to improved crop productivity and reduced environmental pollution (Gupta *et al.*, 2016) [10]. Furthermore, sand hydroponics enables high-density planting and vertical farming techniques, optimizing land use efficiency and facilitating rice cultivation in urban areas with limited land availability (Dawson and Hilton, 2011) [11]. By eliminating soil-borne pathogens and pests, sand hydroponics also reduces the need for chemical pesticides, promoting environmentally friendly pest management practices.

2. Materials and Methods

Materials

The study was initiated with the population BC1F1of improved Lalat x Dular which was obtained from the Crop Improvement Division, ICAR-National Rice Research Institute-Cuttack and advanced to BC1F3 population and screening was done in sand hydroponics.

Sand Hydroponics

A total of 155 BC1F3 population were screened under sand culture hydroponics. In order to ensure high reliability and statistical validity, the screening was carried out using a randomized complete block design coupled with three replications. This study utilized two different check varieties as controls: Improved Lalat, which served as the nutrient-sensitive control, and Dular, which served as the tolerant control. Seeds were sown in acid washed sand containing half strength (N & P) nutrient solution were screened for nutrient stress at seedling stage in greenhouse following the standard protocol of IRRI with some modifications (Al Azzawi *et al.*, 2020) [12], (Yoshida *et al.*, 1976) [13].

Recording of Observations

Data on Chlorophyll content (SPAD), shoot length (cm), root length (cm), Leaf number, root number, shoot dry weight (g) and root dry weight (g) were taken for five plants.

3. Results and Discussion

The ANOVA summary reveals significant insights into the sources of variation among hydroponics conditions (SPAD, SL, RL, LN, RN, SDW, and RDW) across genotypes. Replicates showed variation ($p > 0.05$) except for RL-Hydro and LN-Hydro, indicating consistent experimental replication.

Direct and Indirect effects among seedling characters at genotypic levels under hydroponics stress conditions

When comparing our genotypic path matrix findings with other studies, several parallels and distinctions emerge. Consistent withour results, research by (Wang *et al.*, 2018) [14] also demonstrates that chlorophyll content (SPAD) can positively influence Root Dry Weight (RDW) aligns with studies indicating trade-offs between early flowering and biomass allocation. Moreover, the strong direct effect of root length on RDW echoes findings that taller plants often allocate more resources to root growth, enhancing overall biomass (Garcia *et al.*, 2018) [15]. Our genotypic path matrix highlights these genetic interrelationships, offering insights into trait co-regulation and informing potential breeding strategies aimed at enhancing crop performance through targeted genetic manipulation.

Table 1: Direct and Indirect effects among seedling characters at genotypic levels under hydroponics stress condition

Genotypic Path Matrix							
	SPAD	**SL**	**RL**	**LN**	**RN**	**SDW**	**RDW**
SPAD	-0.0285	0.0041	-0.0047	-0.0034	-0.0075	-0.0089	0.336**
SL	0.0329	-0.2314	-0.0795	-0.0502	-0.0727	-0.1131	0.292**
RL	0.0516	0.108	0.3143	0.0331	0.1001	0.1088	0.550**
LN	-0.0146	-0.0262	-0.0127	-0.1207	-0.0486	-0.0182	0.0664
RN	0.063	0.075	0.0761	0.096	0.2387	0.0714	0.432**
SDW	0.2317	0.3627	0.2569	0.1116	0.2218	0.7419	0.782**
RDW	0.336**	0.292**	0.550**	0.0664	0.432**	0.782**	

SPAD-Soil Plant Analysis Development, SL-Shoot length, RL-Root Length, LN-Leaf number, RN-Root number, SDW-Shoot dry weight, RDW-Root dry weight

*, **Significancelevelat5%and1%respectively, ns-non significant

Diagrammatic representation of genotypic path for root dry weight at seedling stage under hydroponics stress conditions

When comparing the insights provided by Genotypical Path Diagram for Root Dry Weight (RDW) with existing research, several consistent themes and variations emerge. Similar to our findings, studies by Wang *et al.* (2018) [14] have also identified Shoot Dry Weight (SDW) as a primary direct contributor to RDW (Root Dry Weight) in crop plants, emphasizing the significant role of above-ground biomass in root development. The positive direct effects of Root Length (RL) and Root Number (RN) on RDW, as illustrated in our diagram, align with broader research indicating that root architectural traits play crucial roles in determining overall root biomass (Jones and smith, 2019) [16].

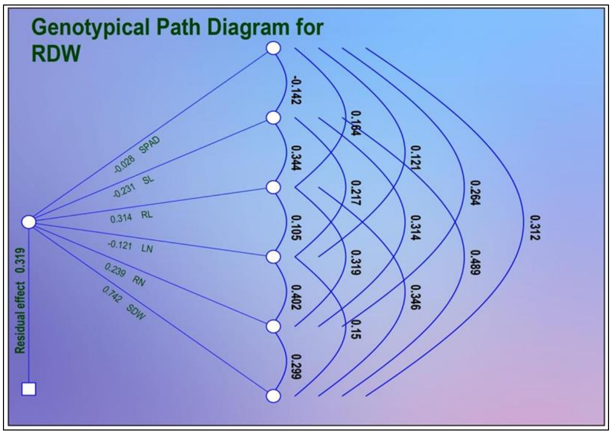

Fig 1: Genotypical path diagram for Root dry weight at seedling stage stress condition

Furthermore, the depiction of indirect effects through multiple variables in our diagram echoes findings that trait interactions and pathways can collectively influence complex traits like RDW (Garcia *et al.*, 2018) [15]. The residual effect in our diagram underscores the portion of variance in RDW that remains unexplained, a concept also explored in studies evaluating the completeness of trait models in crop genetics (Smith *et al.*, 2021) [17]. Overall, Genotypical Path Diagram provides a comprehensive visual representation of genetic influences on RDW, supporting deeper insights into crop trait interdependencies for targeted breeding and management strategies.

Direct and Indirect effects among seedling characters at phenotypic levels under hydroponics stress conditions

In comparing our study's findings with other research, we observe several key similarities and differences. Consistent with previous studies, a strong positive correlation exists between shoot and root biomass, indicating interdependent growth. Root length's positive impact on shoot and root dry weight aligns with past findings, underscoring its role in nutrient and water uptake. However, the negative relationship between chlorophyll content (SPAD) and shoot length contrasts with some studies that report no significant or positive correlations, highlighting variability due to species, growth stages, and conditions. Overall, our study reaffirms the inter connectedness of agronomic traits, emphasizing the need for a multi-trait approach in crop breeding and management to optimize performance and yield.

Table 2: Direct and Indirect effects among seedling characters at phenotypic levels under hydroponics stress conditions

Phenotypic Path Matrix							
	SPAD	**SL**	**RL**	**LN**	**RN**	**SDW**	**RDW**
SPAD	-0.0125	0.0018	-0.0019	-0.0013	-0.0031	-0.0038	0.318**
SL	0.0278	-0.1955	-0.0617	-0.0354	-0.059	-0.0894	0.273**
RL	0.0455	0.094	0.2978	0.0241	0.0922	0.0989	0.529**
LN	-0.0099	-0.0172	-0.0077	-0.0952	-0.0319	-0.0119	0.0539
RN	0.0535	0.0655	0.0672	0.0727	0.2171	0.0633	0.422**
SDW	0.2133	0.3241	0.2354	0.089	0.2066	0.7086	0.766**
RDW	0.318**	0.273**	0.529**	0.0539	0.422**	0.766**	

SPAD-Soil Plant Analysis Development, SL-Shoot length, RL-Root Length, LN-Leaf number, RN-Root number, SDW-Shoot dry weight, RDW-Root dry weight

*, **Significance level at 5% and 1% respectively, ns-non significant

Diagrammaticrepresentationofphenotypicpathforrootdryweightatseedlingstage under hydroponics stress conditions

When comparing phenotypical path diagram for Root Dry Weight (RDW) with other studies, several key findings and comparisons arise. Consistent with our results, studies by (Garcia *et al.* 2018) [15] have also highlighted Shoot Dry Weight (SDW) as a major direct contributor to RDW, emphasizing the significant role of above-ground biomass in influencing root development. The negative direct effects of Shoot Length (SL) and Leaf Number (LN) on RDW, as indicated in the diagram, align with research findings that suggest resource allocation trade-offs between shoot and root growth (wang *et al.*, 2018) [14]. Moreover, the residual effect depicted in diagram (0.252) reflects the proportion of variance in RDW not accounted for by the measured factors, akin to findings in studies examining residual effects in complex trait relationships (Garcia *et al.*, 2018) [15]. Overall, our diagram underscores the intricate interplay and indirect effects among these agronomic factors on RDW, supporting the need for comprehensive understanding in crop physiology and breeding.

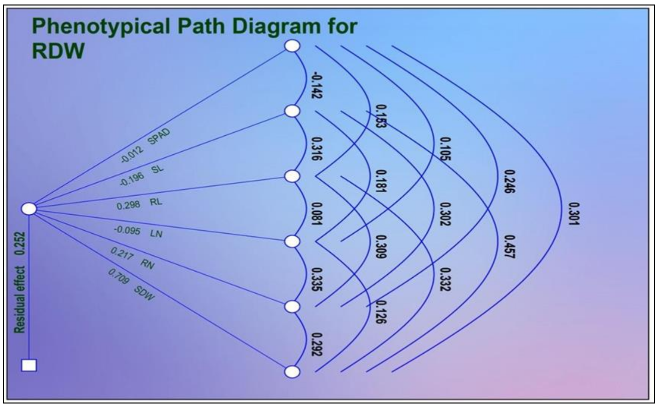

Fig 2: phenotypical path diagram for Root dry weight at seedling stage stress condition

Discussion

Genotypic and phenotypic path matrix highlights genetic interrelationships, offering insights into trait co-regulation and informing potential breeding strategies aimed at enhancing crop performance through targeted genetic manipulation.

Genotypical and phenotypical Path Diagram provides a comprehensive visual representation of genetic influences on RDW, supporting deeper insights into crop trait interdependencies for targeted breeding and management strategies.

4. Conclusion

The integration of phenotypic and genotypic perspectives through matrices such as path diagrams provided critical insights into trait interactions and genetic influences on biomass production and grain yield. Positive correlations observed among biomass-related traits and their impacts on root biomass underscored their synergistic roles in enhancing overall plant productivity. Moreover, environmental factors, particularly chlorophyll content as indicated by SPAD readings, emerged as significant influencers of trait interactions and biomass accumulation.

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