Assessment of drought tolerance in *indica* rice: A new method

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Methodology

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Abstract

Background: Most of the rice-growing areas are exposed to the threat of drought stress due to the effects of climate change. In order to meet this challenge and support an increasing population, it is desirable to breed rice varieties with improved drought tolerance. Breeders' success in developing improved drought resistance lines depends entirely on a simple and accurate screening process. All available drought assessment methods are time consuming, labour oriented and indirect indicators of drought.

Result: We explain a method which efficiently evaluates the drought tolerance degree (DTD) of lowland *indica* rice varieties. DTD is defined as the average of the ratio of green leaf length to the total length of the top three leaves of each rice seedling after drought treatment, and therefore takes a value from 0 to 1. To test the potentiality of the DTD method, 118 doubled haploids with two parents showing different degrees of drought tolerance were assessed, from which the DTD value has been determined. Of the 118 doubled haploids identified by the drought tolerance assessment system under severe drought stress, DH102 showed the strongest drought resistance, with the highest DTD value, and DH22 was found to be the weakest drought resistance with the lowest DTD value. Further correlation analysis revealed a high association of DTD values with RWC, leaf tip drying scores, and leaf rolling scores. Based on these characteristics, the entire population under study was divided into two main groups. PCA analysis suggested three principal components with its first principal component possessing the DTD value, RWC, leaf rolling score, leaf tip drying score, and chlorophyll content index with maximum loading score.

Conclusion: This new method will help in assessing the drought tolerance of *indica* rice varieties. This study indicates the DTD method is simple, cost-effective, direct and relatively accurate for drought-tolerant screening of rice varieties.

Background

Rice is the most commonly consumed staple food in most of the world's population, especially in Asia, accounting for 50–80% of daily caloric intake [1]. Developing countries provide 27% dietary energy and 20% dietary protein [2]. Ideal for areas with high humidity, long exposure to sunlight and assured water supply. The world's population is estimated to grow to 8 billion by 2030, so rice production needs to be increased [3]. Drought is one of the major abiotic stresses, also known as low water stress. Environmental challenges to crop survival and productivity [4]. Modern high-yielding rice varieties are very sensitive to drought stress during the seedling, vegetative and reproductive stage, and even mild drought stress can significantly reduce rice yields [5–7]. Drought affects approximately 23 million hectares of rainfed rice worldwide [8]. This ultimately leads to economic loss for farmers and is considered to be one of the main constraints on limiting rice yields in areas of poor rainwater and irrigation. The development of drought-resistant rice varieties requires at least two factors such as; highly drought-tolerant lowland rice lines/variety/ genotypes used as donor parents, and an effective and suitable evaluation methods for high-throughput screening under field conditions, are required to develop drought-tolerant rice cultivars [9, 10]. Therefore, an evaluation method should be developed for easy and rapid assessment of rice cultivars

for drought tolerance. It is important to start the drought comparative phenotypic screening of breeding material at a very early step of tested lines along with both sensitive and tolerant lines, which would allow a precise monitoring of the applied drought stress level and competitive advantage of the test material versus the promising breeding lines.

In the last decades many screenings method was reported for drought tolerance screening, but most of them are labour consuming, chemical and skilled labour oriented. The physiological trait dependant screening like; chlorophyll content and proline content, serve as the indirect indicators of drought tolerance. The success of drought tolerance breeding depends on the effective and accurate assessment of drought tolerance of rice in the field.

The main purpose of this study was to validate and develop a new screening method that could be used to evaluate rice varieties for drought tolerance in early growth. This method is known as the Drought Tolerance Degree (DTD) and was proposed by Zu [11].

Results

Responses of 118 doubled haploid rice lines to drought stress treatments in drought-tolerance trials

Prior to the drought stress treatments, the 118 doubled haploid (DH) rice lines along with their two parents grew well in earthen pots under the protected net house (Figure 3a), with none showing any aberrant leaf phenotype like rolling or dried leaf tips drying. However, when the soil moisture content dropped to approximately 15% at a depth of 15 cm in the earthen pots (slight drought stress), occurring 7 days after the withholding of water, leaves of the drought-sensitive DH-22 and DH-5 were completely rolled and leaf tips were also dried, whereas the remaining 116 DH lines and the two parents were appeared little or not affected, indicating that DH line- 5 and DH-22 were the most drought sensitive of the 118. When soil moisture content down to 10% (moderate drought stress) (Figure 3b), occurring 12 days after the stopping the watering to the pot, the leaves of DH-134, DH-120, DH-83, DH-75, DH-60, DH-52 were rolled tightly, wilted, or drying, indicating that they were also sensitive to drought stress. When the soil moisture content dropped down to about 5-6%, (severe drought stress), occurring 15 days after the withholding of water, three leaves on the top, of most of the cultivars showed extensive damage (Figure 3c). Under this condition, DH-5 and DH-22 was completely dead, whereas, leaves of DH-44, DH-95, DH-102 and DH-126 showed less damage, indicating that they are less sensitive to drought stress. By comparison, for leaf rolling, leaf tip drying scoring and RWC of all the DH lines, only DH-44, DH-95 DH-102and DH126 had higher RWC value with lower score of leaf rolling and leaf tip drying scoring, suggesting that they are strongly drought-tolerant cultivars.

DTD values after severe drought treatment

As the DTD value is actually the mean of the ratios of the green to total length of the top three leaves, a higher DTD value reciprocate to less leaf damage caused by drought stress and thus to stronger drought

tolerance, whereas a lower DTD value correlate more leaf damage and weaker drought tolerance. As shown in Table 1, all the plants of the two parents under control had DTD values of approximately 0.99, implying that for these well-watered plants there was very little drying of leaf tips. After the severe drought treatments, DH-102 had the highest DTD value (0.993) followed by DH-44 (0.992), DH-126 (0.99) and DH-95 (0.99) among the 118 DH lines and the 2 parents, but DH-22 had the lowest (0.006), which is also lowest than the susceptible parent IR-20. The results were consistent with their drought tolerance as observed in the drought-tolerance trials. The tolerant line Mahulata, which is a farmer's variety, is well adapted to field drought conditions. The DTD of Mahulata (drought treated) was 0.639, which was 0.354 lower than that of DH-102, again indicating that DH-102 was very tolerant to the severe drought treatment (Table 1). In fact, Mahulata was found more sensitive than DH-102 in the drought-tolerance trials, with a result consistent with the DTD values. The DTD value of DH-5 and DH-22 were 0.0068 and 0.0065 respectively, with the former 0.0072 lower and the later was 0.0075 lower than the DTD value of the susceptible check, IR-20 (0.014). The DTD value of the drought susceptible parent IR-20 was found to be 0.014 and for tolerant parent i.e., Mahulata was 0.639, whereas the DTD value of the well-watered condition of these two parents were found to be 0.989 and 0.93 respectively (Figure 3d). These findings were also in accordance with the leaf rolling and leaf tip drying score of drought screening. Based on these findings, it was confirmed that DTD value reflect the extent of drought tolerance among the DH lines.

Table 1
DTD value of all the lines along with control

Genotype Name	Mean DTD	SD (+/-)	Genotype Name	Mean DTD	SD (+/-)	Genotype Name	Mean DTD	SD (+/-)
DH1	0.663	0.130	**DH54**	0.856	0.007	**DH100**	0.298	0.002
DH3	0.548	0.030	**DH56**	0.424	0.099	**DH101**	0.989	0.007
DH4	0.419	0.050	**DH57**	0.761	0.022	**DH102**	0.994	0.001
DH5	0.007	0.000	**DH58**	0.679	0.018	**DH104**	0.182	0.080
DH6	0.232	0.041	**DH59**	0.948	0.021	**DH106**	0.522	0.102
DH7	0.458	0.133	**DH60**	0.016	0.003	**DH107**	0.770	0.005
DH8	0.639	0.121	**DH62**	0.057	0.004	**DH108**	0.607	0.083
DH9	0.198	0.035	**DH64**	0.982	0.006	**DH109**	0.313	0.212
DH10	0.910	0.077	**DH65**	0.344	0.182	**DH110**	0.098	0.007
DH12	0.988	0.007	**DH66**	0.087	0.002	**DH113**	0.952	0.049
DH13	0.989	0.005	**DH67**	0.130	0.020	**DH114**	0.363	0.215
DH14	0.981	0.009	**DH69**	0.663	0.153	**DH115**	0.680	0.046
DH15	0.656	0.205	**DH70**	0.663	0.153	**DH116**	0.635	0.023
DH16	0.617	0.220	**DH71**	0.680	0.098	**DH117**	0.586	0.305
DH17	0.186	0.081	**DH73**	0.073	0.014	**DH118**	0.123	0.023
DH18	0.288	0.079	**DH74**	0.544	0.052	**DH119**	0.056	0.012
DH20	0.989	0.005	**DH75**	0.086	0.003	**DH120**	0.046	0.009
DH22	0.007	0.001	**DH76**	0.496	0.001	**DH121**	0.380	0.046
DH25	0.285	0.055	**DH77**	0.388	0.071	**DH122**	0.060	0.020
DH28	0.574	0.118	**DH78**	0.550	0.045	**DH123**	0.084	0.024
DH29	0.623	0.006	**DH79**	0.059	0.024	**DH124**	0.509	0.251
DH30	0.507	0.029	**DH80**	0.983	0.003	**DH125**	0.500	0.129
DH31	0.671	0.003	**DH81**	0.286	0.124	**DH126**	0.991	0.000
DH32	0.542	0.010	**DH82**	0.710	0.133	**DH127**	0.988	0.002
DH34	0.740	0.011	**DH83**	0.154	0.022	**DH128**	0.630	0.106
DH36	0.197	0.065	**DH84**	0.238	0.013	**DH129**	0.662	0.045

Genotype Name	Mean DTD	SD (+/-)	Genotype Name	Mean DTD	SD (+/-)	Genotype Name	Mean DTD	SD (+/-)
DH37	0.055	0.001	**DH85**	0.273	0.113	**DH130**	0.869	0.051
DH38	0.563	0.044	**DH86**	0.099	0.009	**DH131**	0.547	0.034
DH39	0.552	0.007	**DH87**	0.267	0.044	**DH132**	0.240	0.104
DH40	0.384	0.062	**DH88**	0.250	0.118	**DH133**	0.516	0.128
DH41	0.988	0.007	**DH89**	0.836	0.093	**DH134**	0.022	0.004
DH43	0.772	0.045	**DH90**	0.983	0.004	**DH135**	0.072	0.017
DH44	0.992	0.000	**DH91**	0.286	0.082	**DH136**	0.626	0.305
DH45	0.397	0.084	**DH94**	0.625	0.038	**DH137**	0.986	0.007
DH46	0.150	0.079	**DH95**	0.990	0.002	**Mahulata**	0.639	0.033
DH48	0.269	0.182	**DH96**	0.056	0.058	**IR20**	0.014	0.007
DH50	0.098	0.012	**DH97**	0.202	0.010	**Mahulata(Control)**	0.993	0.003
DH51	0.994	0.000	**DH98**	0.122	0.037	**IR20(Control)**	0.989	0.004
DH52	0.020	0.002	**DH99**	0.092	0.007			

The Correlation of DTD values with physiological traits

Previous study has demonstrated that a few physiological traits are well correspondence with drought tolerance in rice [12]. DTD have a very strong positive correlation (Table 2, Figure 4) with RWC (0.771), whereas it was very strongly negatively correlated with leaf drying scoring (-0.778) and leaf rolling scoring (-0.850). DTD also positively correlated with chlorophyll content index (0.526) and leaf number (0.447) & negatively correlated with leaf canopy temperature (-0.405) and tiller number (-0.465). The RWC again very strongly negatively correlated with leaf drying scoring (-0.753) and leaf rolling scoring (-0.788). There was a very strong positive correlation between leaf drying scoring and leaf rolling scoring (0.826).

Table 2: Correlation of DTD with other morpho-physiological traits

	LDS	LCT	CCI	LRS	LN	TN	LA	RWC	DTD	PH
LDS	1	0.28005*	-0.52242*	0.82642**	-0.49793*	0.26403*	-0.23101*	-0.75324**	-0.7785**	-0.06398
LCT		1	-0.24507*	0.40989*	-0.31919*	0.14586	-0.08317	-0.29677*	-0.4056*	-0.01496
CCI			1	-0.56678*	0.32372*	-0.25214*	0.039029	0.5489*	0.52619*	0.048541
LRS				1	-0.53969*	0.35035*	-0.27265*	-0.78802**	-0.85088**	-0.00872
LN					1	0.24395*	0.30444*	0.43929*	0.44776*	0.19921
TN						1	0.077606	-0.32479*	-0.46501*	0.000387
LA							1	0.10226	0.14855	0.47744*
RWC								1	0.77171**	-0.15177
DTD									1	0.056831
PH										1

LDS: Leaf drying scoring, LCT: Leaf canopy temperature(^{0}C), CCI: Chlorophyll content index, LRS: Leaf rolling scoring, LN: leaf number, TN: Tiller number, LA; Leaf area (cm^2), RWC: Relative water content, DTD: Drought tolerance degree, PH: Plant height(cm)

Principal component analysis (PCA) of DTD value along with other physiological traits

Among the multivariate analysis techniques, PCA is the most frequently used because it minimizes the dimensionality of the data and provides component scores capturing the variation in the multivariate analysis [13, 14]. The principal component analysis was done, which gave us three major principal components (PCs) i.e., PC1, PC2 and PC3 based on an Eigenvalue greater than equal to 1. Further, PC1 covered 45.07% of total variability whereas that of PC2 covered 16.54% and PC3 covered 11.09% of total variance. The cumulative variance observed by these three PCs was 72.71% (Table 3 and Figure 5). The variability contribution of each variable under consideration, towards PC1 was given in figure 6. PC1 was the most important major PC based on the highest Eigen value (Table 2). The loading plot figure (Figure 6) indicate that DTD had the major contribution towards the PC1 followed by RWC and chlorophyll content index. The bi-plot of the PCA (Figure 7) explain the traits DTD and RWC along with chlorophyll content index were highly correlated with each other and can be used for drought screening of the lines. On the other hand, the angle developed by leaf rolling scoring and leaf drying scoring with DTD value suggesting a negative correlation among these traits. Again, the above two traits clearly differentiate the drought tolerant parent, Mahulata and susceptible parent, IR-20 from each other with an indication that the DTD value can be indirectly used for drought tolerance screening.

Table 3
Principal component and their eigen value (Major principal components are bold)

PC	Eigenvalue	% Variance	Cumulative variance
1	**4.50715**	**45.071**	**45.071**
2	**1.65445**	**16.545**	**61.616**
3	**1.10941**	**11.094**	**72.71**
4	0.835506	8.3551	81.0651
5	0.649676	6.4968	87.5619
6	0.469732	4.6973	92.2592
7	0.281541	2.8154	95.0746
8	0.213713	2.1371	97.2117
9	0.166848	1.6685	98.8802
10	0.111972	1.1197	99.9999

2.5 Hierarchical clustering analysis

The cluster analysis of 118 doubled haploid lines along with 2 parents both in drought treatment and controlled condition was also performed to confirm the results obtained in PCA. The two major clusters were formed with a dissimilarity co-efficient of 380 that divide the entire population under the experiment into two clusters (Figure 8). The cluster-I covered all the 43 lines including the both parent in controlled condition. Most of the tolerant lines were grouped under this cluster-I. Cluster-II covered all other 79 lines including both of the parents under drought treatment.

Discussion

The correspondence of DTD values with physiological traits

Leaf tip drying scoring and leaf rolling scoring are given with a higher value for lower degree of drought tolerance [15]. Leaf rolling can help in maintaining internal plant water status [16]. RWC is directly proportional to the degree of drought tolerance of a cultivar [17]. The DTD value in the experiment was positively correlated with RWC and negatively correlated with leaf rolling and leaf tip-drying scoring. Again, chlorophyll content index of leaf and leaf canopy temperature that are a strong indicator of degree of drought tolerance were inversely proportional to each other, because of the reason that, the live cell containing chlorophyll maintain the cell temperature through transpiration therefore the tolerant line has lower canopy temperature with higher chlorophyll content [18–20]. Here DTD value is positively correlated

with chlorophyll content index and inversely with canopy temperature. All these evidences pointing out that, the DTD value can be used to screen the drought tolerance degree of cultivars and it is directly proportional to the drought tolerance degree.

Roles of the DTD method in drought-tolerance breeding

Previously known that morpho-physiological traits of crops can either contribute to drought tolerance or become indicators of the crops' response to drought stress [21, 22]. Our results demonstrated that the DTD values are roughly consistent with physiological parameters, such that higher DTD values corresponded to higher water potential, RWC and chlorophyll contents as well as lower Leaf canopy temperature content. The DTD values can be used for rough estimates of the levels of these traits, making it useful for the study of drought tolerance. Based on this study, the DTD values can serve as important reference points for selecting drought-tolerant cultivars or lines.

Advantages and disadvantages of the DTD method over other evaluation approaches in drought-tolerance breeding of rice

In the recent past, many methods for evaluating rice drought tolerance have been developed, including leaf rolling, survival rate, seed setting rate, grain yield, RWC, days to harvest, Abscisic acid (ABA) and proline contents [22–31]. Screening at a very early step will accelerate the development of tolerant lines [32]. Screening can be done at the germination stage with the help of PEG (polyethylene glycol) [33]. Screening in seedling stage can also be performed [34]. However, all of these methods either take a long time, producing unexpected results, or are indirect and inaccurate. Some of the method like PEG mediated or proline content mediated screening involved in utilization of the prescribed chemical for screening. The only early response to drought stress is reflected by leaf rolling and is cultivar-dependent. Survival rate, seed setting rate, and grain yield have been used to evaluate drought tolerance [35, 36]. However, they are influenced by many other environmental factors, including high or low temperature and disease other than drought, making them poor indicators of drought tolerance. Here, we have demonstrated the DTD method used for evaluation of drought tolerance in lowland rice cultivars that can assess the drought tolerance in rice cultivars in a very simple and highly efficient manner. This method allows identification of the drought tolerance of lowland rice at the seedling stage, and accordingly many other unexpected environmental factors influencing plant development are avoided and the use of chemical can also be completely avoided. In addition, this method of screening can lower down deviations caused by the intrinsic differences in leaf rolling among different cultivars. Thus, the DTD method appears to overcome the disadvantages of previous evaluation methods and to produce more accurate results. As the DTD method is based on simple measurements of leaf length after severe drought stress at seedling stage, so there is no need of skilled personals for data recording. The DTD values can be compared in quantitative terms, making this method not only relatively easy to handle but also useful for differentiating degrees of drought tolerance among cultivars. As no special apparatus is required for the DTD method, it can be easily applied in drought-tolerance breeding. In addition, the approach can be extended to identify drought tolerance in lowland rice cultivars. Based on the outcomes obtained in this study, it seems that

the DTD method is a very promising method for drought-tolerance breeding and can be considered to accelerate the drought tolerance-breeding programme in near future.

The one and only major drawback in this method of screening was, this method is not applicable for evaluating drought tolerance degree of rice at harvest stage, as natural maturity and senescence will produce brown leaves which can make confusion and skewed the graph towards drought susceptibility.

Conclusion

In conclusion, a DTD method for screening drought tolerance in indica rice was developed which will certainly help the rice researchers to move fast in identifying drought tolerant lines. In this study, the physiological traits of 118 Doubled haploids along with the parents, were positively correlated with the DTD values. As compared to other methods, DTD method is found to be cost effective which involves the drought tolerance of low land rice at the vegetative stage under harsh drought stress condition. Besides, this established method is different from the previous one reported in *japonica* sub species on certain key points: a) *indica* rice b) vegetative stage drought tolerance c) physiological parameters such as RWC, chlorophyll content index, tiller number, leaf tip drying score, leaf rolling score, leaf area, leaf canopy temperature, leaf number and plant height d) pot culture e) 118 doubled haploid mapping population along with parents.

This method is found to be efficient in wide utilisation for drought tolerance screening due to its cost-effectiveness.

Methods

Plant materials and methods

The experiment was conducted in net house at ICAR-National rice research institute, Cuttack, Odisha, India (20°27'9"N, 85°56'25"E). A total of 118 doubled haploid developed from the F_1s of IR-20 (Drought susceptible parent) and Mahulata (Drought tolerant parent) through androgenesis along with the two parents were planted in earthen pot in the net house. Both the parents were taken as control for the experiment. The earthen pots with 30cm long and 30cm diameter were filled with homogenised NRRI lowland farm soil and the holes at the bottom were sealed not to allowing the drainage. The lower portion (80%) of the pots was filled with a bulk density equivalent to 1.15 g/cm3 of dry soil by compacting the soil even after every 5 cm during the filling process [37]. To settle down the soil, the pots were saturated with water for a few days before transplanting the seedlings. The upper (20%) portion was filled with lowland irrigated soil. The soil level was set aside 5 cm below the edge of the pots. All the planting materials were replicated three times to check the environmental effect. The net hose was fully protected to ensure that not a single drop of rain could fall on the pot during the study.

Drought stress treatment

After transplanting, all pots were irrigated every alternate day until the onset of drought. Soil moisture content after full irrigation was approximately 56% (v/v) at a depth of 20 cm as determined by moisture measurement (MM). Drought stress was initiated on the 12nd day after transplanting by stopping the watering and soil moisture was monitored in alternative days by MM. Soil water potential at a depth of 15 cm was recorded on alternate days through tensiometer tubes (Soil moisture Equipment Co.) placed randomly in five to six locations in the stress as well as controlled pots. The drought treatments were not stopped until leaves of the tolerant parent, Mahulata displayed marked differences in leaf damage. The drought treatment was carried out to check some transgressive segregants in relation to tolerant parent.

Data recording

Soil moisture and tensiometer reading were taken in alternative day. Leaf tip drying and leaf rolling scoring was recorded as per scoring method of the Standard Evaluation System (SES) [38]. Leaf canopy temperature was measured using an Infra-red thermometer (IR-thermometer) (Model IR50, Spot Infrared Thermometer, Spectrum Instrument Ltd.) and leaf chlorophyll content index was recorded using SPAD502. The relative water content (RWC) was determined according to the method of Matin [17] and was collected at 2 pm on the last day drought treatment. The leaf area was calculated by the formula; Leaf area = leaf length x width x 0.71 [39] and expressed in cm^2. Apart from this plant height, the number of leaves and the number of tillers we recorded. All the data was taken on the last day of drought treatment.

Calculation of DTD

DTD is defined as the average ratio of green leaf length to total leaf length of the top three leaves of each plant after severe drought treatment [11] (Figure 1). The DTD value varies primarily between 0 and 1. The green leaf length and total leaf length of the first leaf are identified as F1 and F2, respectively. Similarly, the green leaf length and total leaf length of the second leaf are separately called S1 and S2, and those of the third leaf are called T1 and T2. The untreated control varieties were treated in the same way to obtain DTD values. The DTD value for each material was calculated using the following formula:

$$Xj = \frac{1}{n}\overset{n}{?}_{i=1}[\left(\frac{F1}{F2} + \frac{S1}{S2} + \frac{T1}{T2}\right)/3]$$

DTD value= $(X_1+X_2+X_3)/3$

where, n is the number of measured plants in each replicate, Xj represents one of the three replicates DTD value in each cultivar, X_1, X_2, X_3 denote replicate I, replicate II, and replicate III, respectively.

Statistical analysis

All the data collected were subjected to analysis of PCA, correlation study and hierarchical analysis by using PAST 4.03.

Abbreviations

DTD: Drought tolerance degree, DH: Doubled haploid, RWC: Relative water content, PCA: Principal component analysis, PC: Principal component, PEG: Polyethylene glycol, ABA: Abscisic acid, MM: Moisture meter, SES: Standard evaluation system, IR-thermometer: Infra-red thermometer.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

SKS, JLK, PC, SS conceptualized methodology. SKS, KJ, JLK designed and conducted the experiment. SKS, KJ, JLK, PC Collection of data. SKS, PC, PNJ, DNB analyse the data SS, PC, SKS, PNJ, DNB manuscript preparation. SS, SKS, PC, JLK performed the analysis of the experimental results.

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References

1. Khush GS. What it will take to feed 5.0 billion rice consumers in 2030. Plant Mol Biol. 2005 Sep;59(1):1–6.
2. Kumar S, Dwivedi SK, Haris AA, Prakash V, Mondal S, Singh SK. Screening and identification of rice genotypes for drought tolerance at reproductive stage under rainfed lowland condition. Journal of AgriSearch. 2015 Jun 1;2(2):105-11.
3. Villa JE, Henry A, Xie F, Serraj R. Hybrid rice performance in environments of increasing drought severity. Field Crops Research. 2012 Jan;18:125:14–24.
4. Shashidhar HE, Kanbar A, Toorchi M, Raveendra GM, Kundur P, Vimarsha HS, Soman R, Kumar NG, Bekele BD, Bhavani P. Breeding for drought resistance using whole plant architecture—conventional and molecular approach. Plant breeding from laboratories to Fields. Vienna, Austria. 2013 May p. 22.
5. O'toole JC. Adaptation of rice environments. Drought Resistance in Crops with Emphasis on Rice; Paddyfield: Manila, Philippines. 1982:195-213.
6. Torres RO, Henry A. Yield stability of selected rice breeding lines and donors across conditions of mild to moderately severe drought stress. Field crops research. 2018 May 1;220:37-45.
7. Swain P, Raman A, Singh SP, Kumar A. Breeding drought tolerant rice for shallow rainfed ecosystem of eastern India. Field Crops Research. 2017 Aug;1:209:168–78.
8. Serraj R, McNally KL, Slamet-Loedin I, Kohli A, Haefele SM, Atlin G, Kumar A. Drought resistance improvement in rice: an integrated genetic and resource management strategy. Plant Production Science. 2011 Jan 1;14(1):1-4.
9. Kondo M, Murty MV, Aragones DV. Characteristics of root growth and water uptake from soil in upland rice and maize under water stress. Soil Science and Plant Nutrition. 2000 Sep 1;46(3):721-32.
10. Samejima H, Babiker AG, Mustafa A, Sugimoto Y. Identification of Striga hermonthica-resistant upland rice varieties in Sudan and their resistance phenotypes. Front Plant Sci. 2016 May;13:7:634.
11. Zu X, Lu Y, Wang Q, Chu P, Miao W, Wang H, La H. A new method for evaluating the drought tolerance of upland rice cultivars. The Crop Journal. 2017 Dec 1;5(6):488-98.
12. Zhu JK. Salt and drought stress signal transduction in plants. Annual Rev plant biology. 2002 Jun;53(1):247–73.
13. Leardi R. Chemometrics. From classical to genetic algorithms. Grasas y Aceites. 2002 Mar 30;53(1):115–27.
14. Siebert KJ. Chemometrics in brewing—A review. Journal of the American Society of Brewing Chemists. 2001 Sep 1;59(4):147-56.
15. Singh M, Jain M, Pant RC. Clonal variability in photosynthetic and growth characteristics of Populus deltoides under saline irrigation. Photosynthetica. 2000 Jan;36(4):605–9.
16. Gana AS. Screening and resistance of traditional and improved cultivars of rice to drought stress at Badeggi, Niger State, Nigeria. Agric Biology J North Am. 2011;2(6):1027–31.

17. Matin MA, Brown JH, Ferguson H. Leaf water potential, relative water content, and diffusive resistance as screening techniques for drought resistance in barley. Agron J. 1989 Jan;81(1):100–5.
18. Dingkuhn M, De Datta SK, Dorffling K, Javellana C, Datta S. Varietal differences in leaf water potential, leaf net CO2 assimilation, conductivity and water use efficiency in upland rice. Aust J Agric Res. 1989;40(6):1183–92.
19. Blum A. Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. Plant Cell Environ. 2017 Jan;40(1):4–10.
20. Li J, Li Y, Yin Z, Jiang J, Zhang M, Guo X, Ye Z, Zhao Y, Xiong H, Zhang Z, Shao Y. Os ASR 5 enhances drought tolerance through a stomatal closure pathway associated with ABA and H2O2 signalling in rice. Plant Biotechnol J. 2017 Feb;15(2):183–96.
21. Liu P, Xu ZS, Pan-Pan L, Hu D, Chen M, Li LC, Ma YZ. A wheat PI4K gene whose product possesses threonine autophophorylation activity confers tolerance to drought and salt in Arabidopsis. Journal of experimental botany. 2013 Jul 1;64(10):2915-27.
22. Sharma S, Villamor JG, Verslues PE. Essential role of tissue-specific proline synthesis and catabolism in growth and redox balance at low water potential. Plant Physiol. 2011 Sep;157(1):292–304.
23. Akbar MR, Purwoko BS, Dewi IS, Suwarno WB. Agronomic and drought tolerance evaluation of doubled haploid rice breeding lines derived from anther culture. SABRAO Journal of Breeding & Genetics. 2018 Jun 1;50(2).
24. Huang Q, Wang Y. Overexpression of TaNAC2D displays opposite responses to abiotic stresses between seedling and mature stage of transgenic Arabidopsis. Front Plant Sci. 2016 Nov;23:7:1754.
25. Cheng MC, Liao PM, Kuo WW, Lin TP. The Arabidopsis ETHYLENE RESPONSE FACTOR1 regulates abiotic stress-responsive gene expression by binding to different cis-acting elements in response to different stress signals. Plant Physiol. 2013 Jul;162(3):1566–82.
26. Hossain MM, Liu X, Qi X, Lam HM, Zhang J. Differences between soybean genotypes in physiological response to sequential soil drying and rewetting. The Crop Journal. 2014 Dec 1;2(6):366-80.
27. Ashfaq M, Haider MS, Khan AS, Allah SU. Breeding potential of the basmati rice germplasm under water stress condition. Afr J Biotechnol. 2012;11(25):6647–57.
28. Bunnag S, Pongthai P. Selection of rice (Oryza sativa L.) cultivars tolerant to drought stress at the vegetative stage under field conditions. American Journal of Plant Sciences. 2013 Aug 21;4(09):1701.
29. Sokoto MB. Response of rice varieties to water stress in Sokoto, Sudan Savannah, Nigeria. J Biosci Med. 2014 Mar;6(01):68. 2(.
30. Dewi IS, Trilaksana AC, Koesoemaningtyas T, Purwoko BS. Karakterisasi galur haploid ganda hasil kultur antera padi. Buletin Plasma Nutfah Vol. 2009;15(1).
31. Salunkhe AS, Poornima R, Prince K, Kanagaraj P, Sheeba JA, Amudha K, Suji KK, Senthil A, Babu RC. Fine mapping QTL for drought resistance traits in rice (Oryza sativa L.) using bulk segregant

analysis. Mol Biotechnol. 2011 Sep;49(1):90–5.

32. Boopathi NM, Swapnashri G, Kavitha P, Sathish S, Nithya R, Ratnam W, Kumar A. Evaluation and bulked segregant analysis of major yield QTL qtl12. 1 introgressed into indigenous elite line for low water availability under water stress. Rice Science. 2013 Jan 1;20(1):25-30.
33. Widyastuti Y, Purwoko BS, Dewi IS. Combining ability studies using line× tester analysis for germination traits in hybrid rice under stress conditions. SABRAO Journal of Breeding & Genetics. 2017 Sep 1;49(3).
34. Verulkar SB, Verma SK. Screening protocols in breeding for drought tolerance in rice. Agricultural Res. 2014 Mar;3(1):32–40.
35. You J, Zong W, Hu H, Li X, Xiao J, Xiong L. A STRESS-RESPONSIVE NAC1-regulated protein phosphatase gene rice protein phosphatase18 modulates drought and oxidative stress tolerance through abscisic acid-independent reactive oxygen species scavenging in rice. Plant Physiol. 2014 Dec;166(4):2100–14.
36. Darkwa K, Ambachew D, Mohammed H, Asfaw A, Blair MW. Evaluation of common bean (Phaseolus vulgaris L.) genotypes for drought stress adaptation in Ethiopia. The crop journal. 2016 Oct 1;4(5):367-76.
37. Bernier J, Serraj R, Kumar A, Venuprasad R, Impa S, RP VG, Oane R, Spaner D, Atlin G. The large-effect drought-resistance QTL qtl12. 1 increases water uptake in upland rice. Field Crops Research. 2009 Feb;10(2):139–46. 110(.
38. International Rice Research Institute (IRRI). 2013. Standard evaluation system for rice, 5th ed.
39. Yoshida S, Parao FT. Climatic influence on yield and yield components of lowland rice in the tropics. Clim rice. 1976;20:471–94.

Figures

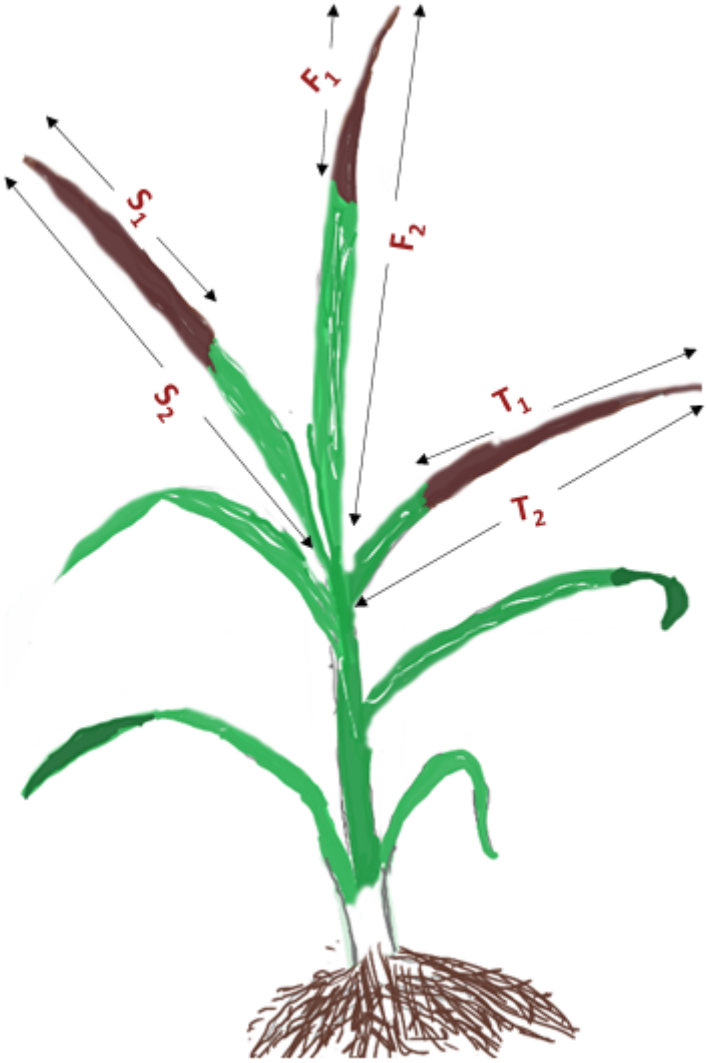

Figure 1

A schematic diagram of parts of leaves used to calculate DTD values. F1, S1, and T1 represent the lengths of the green parts of the first, second, and third leaves, respectively, whereas F2, S2, and T2 denote the entire lengths of the first, second, and third leaves, respectively. The dried parts of leaves resulting from drought stress are shown in brown.

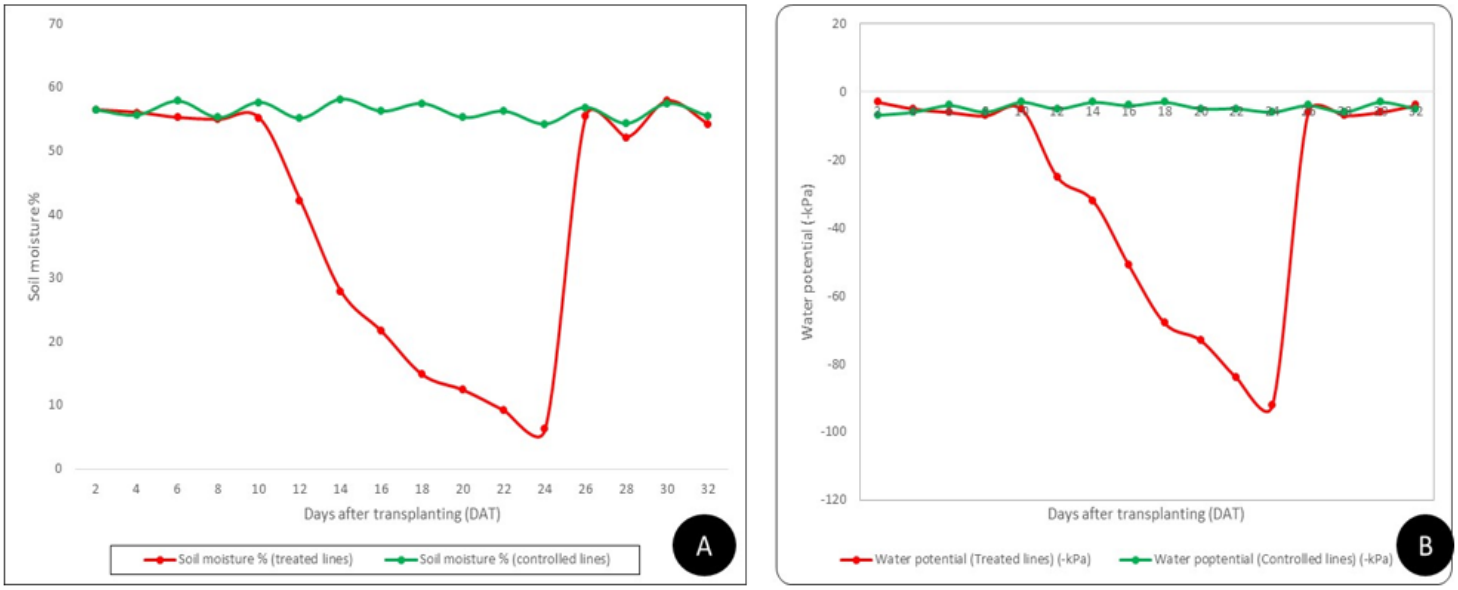

Figure 2

A) Soil moisture and **B)** soil water potential reading of the tested population. Soil moisture level at 20cm below ground measured on every alternative day with the help of Moisture meter. Soil water potential at a depth of 15 cm below ground level recorded on alternate days through tensiometer tubes (Soil moisture Equipment Co.)

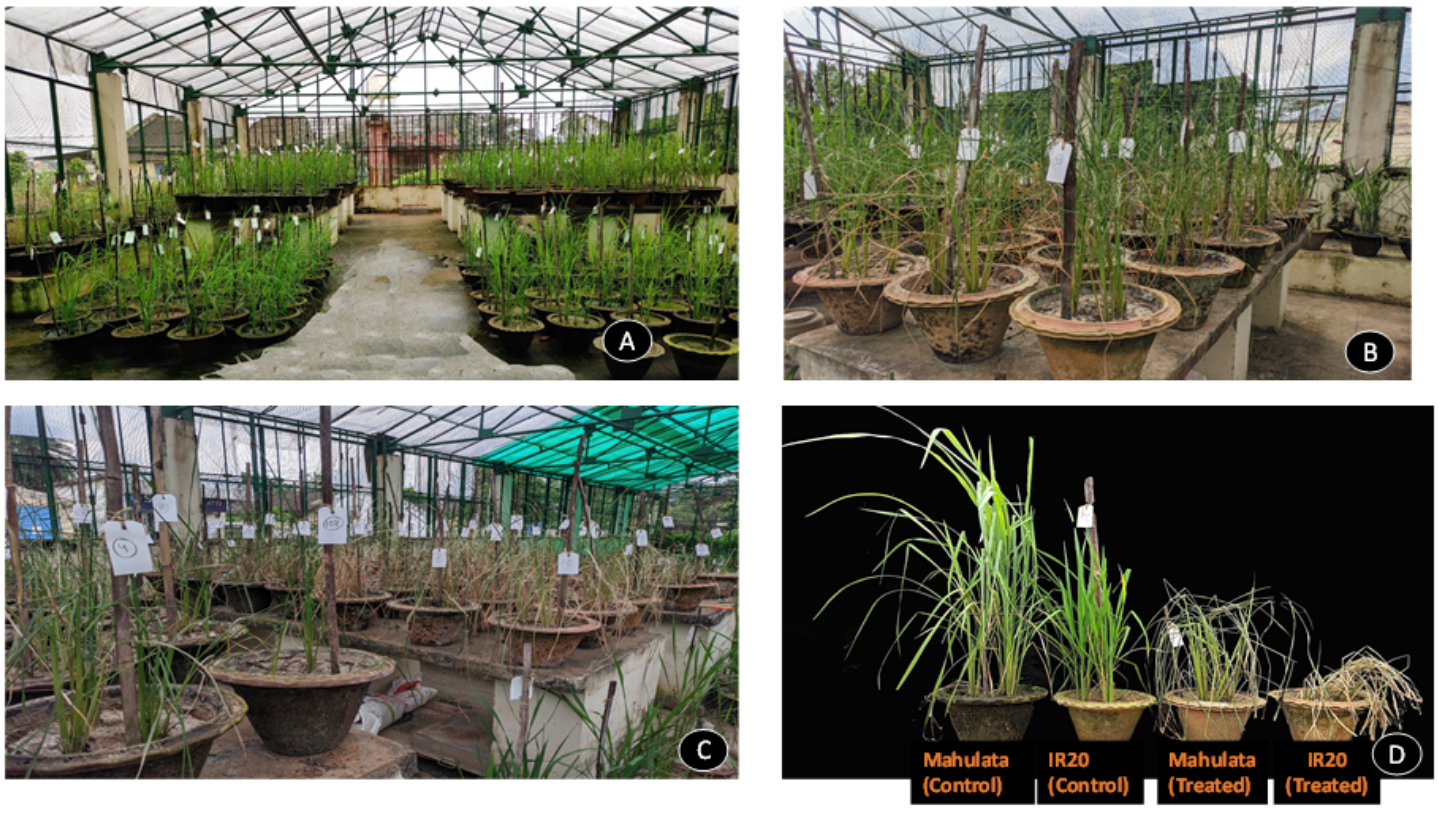

Figure 3

Response of 118 doubled haploid and its parents to different water condition. **A)** performance of the experimental planting material under well-watered condition **B and C)** Response of the experimental planting material on the final day of drought treatment **D)** Comparative response of parents on the last day of drought treatment under controlled (well-watered) and treated (drought treatment) condition.

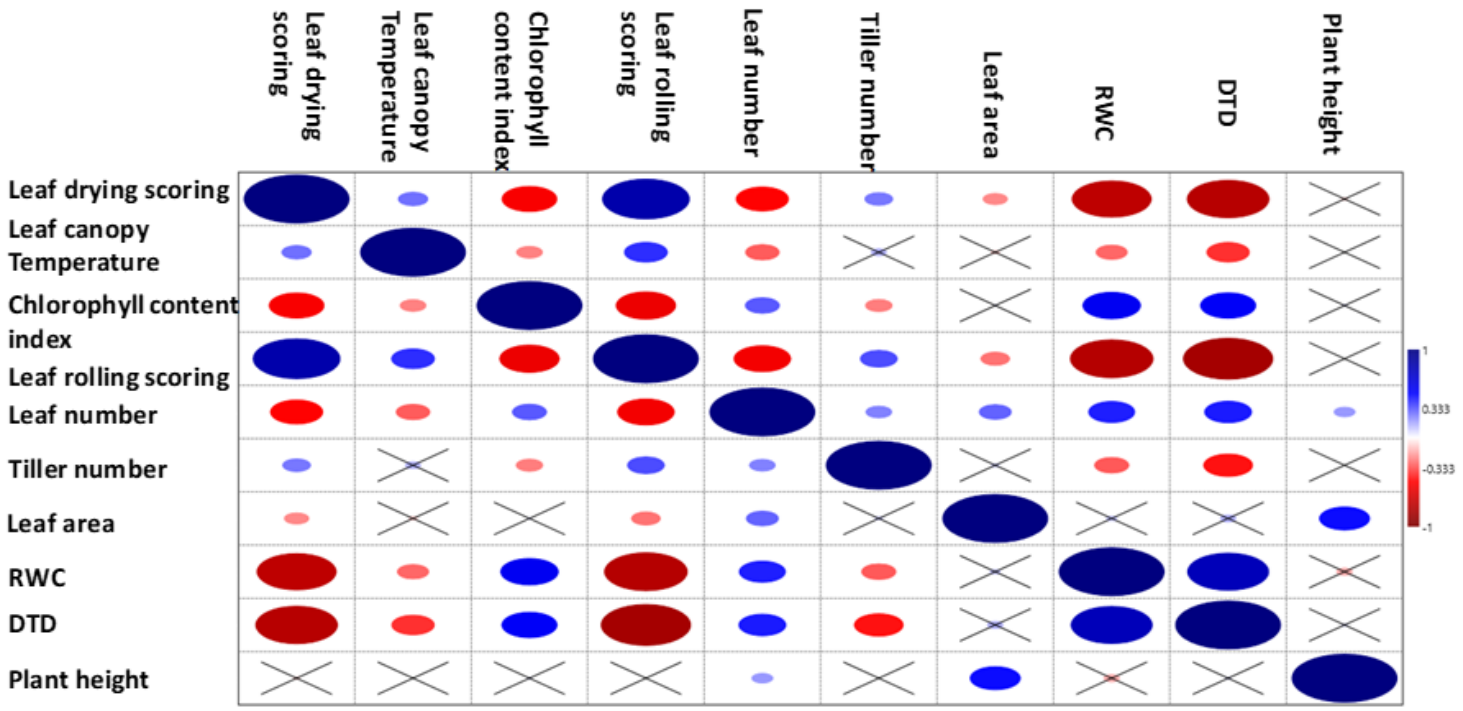

Figure 4

Schematic representation of correlation study among the different traits under testing

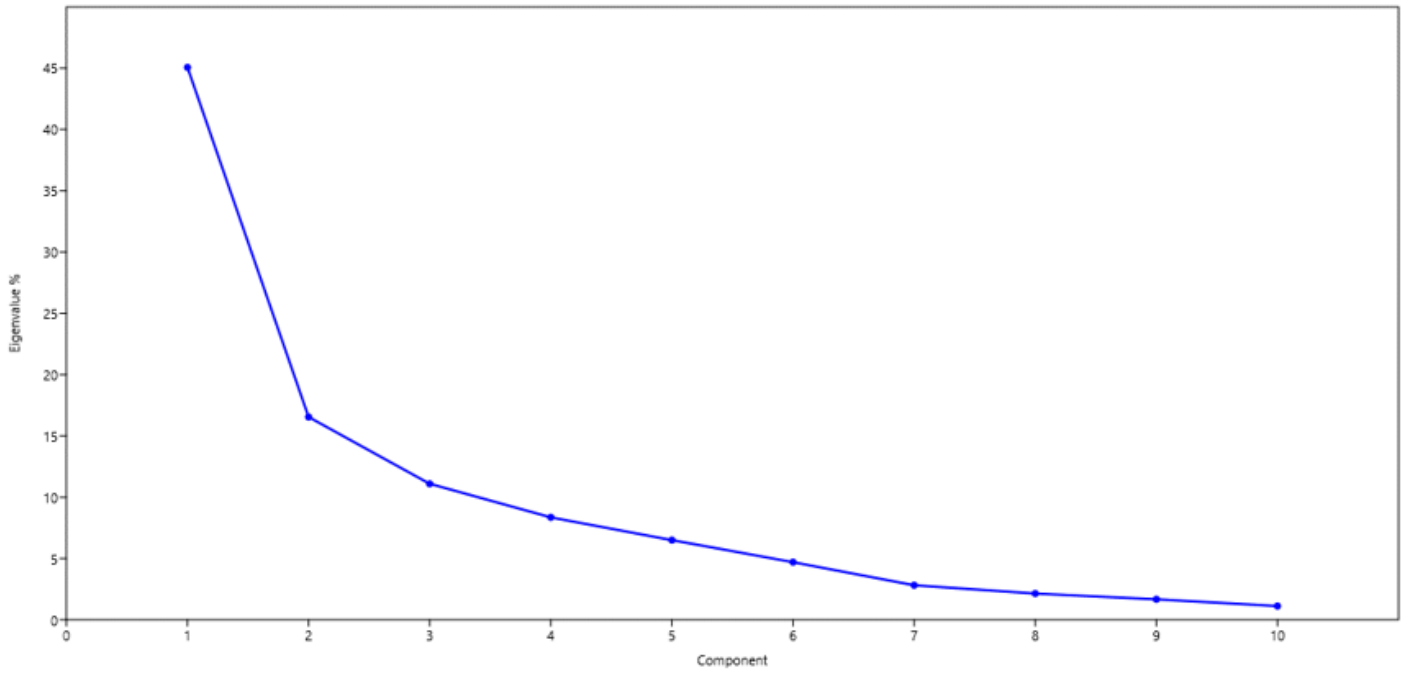

Figure 5

Scree plot analysis of principal components

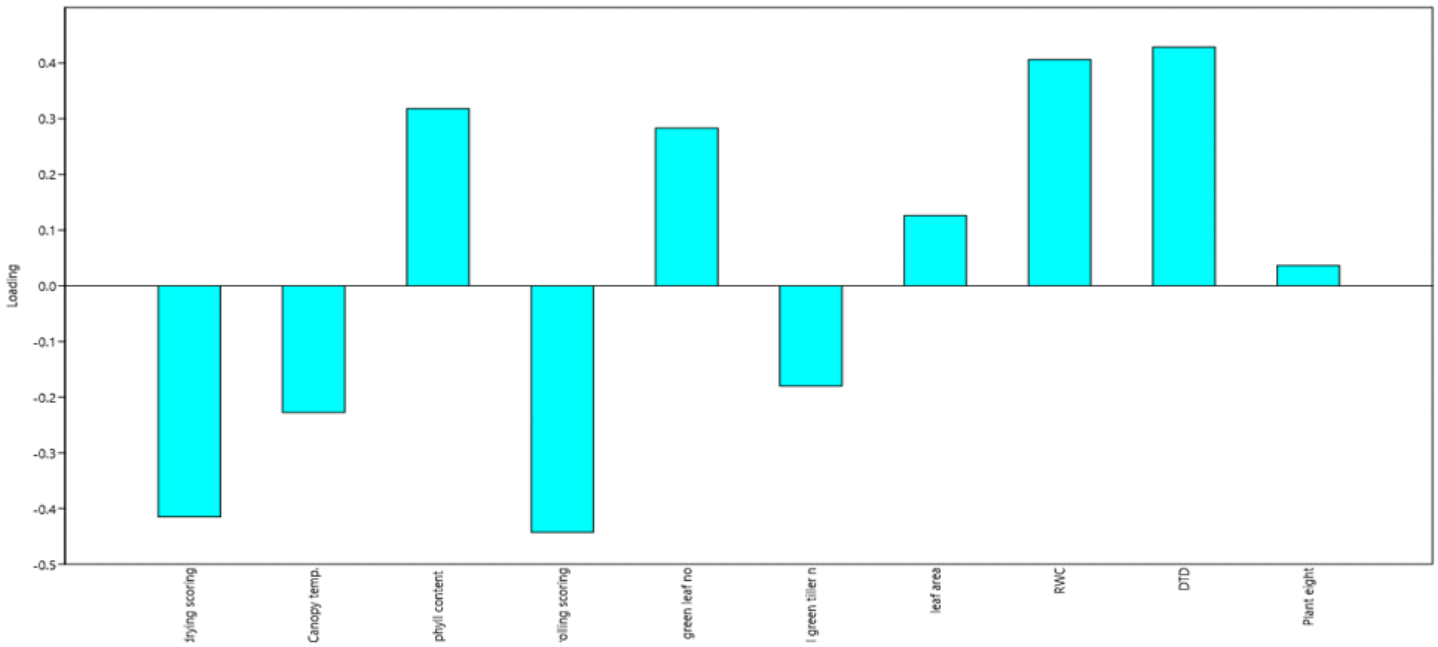

Figure 6

Loading plot of different factors under principal component analysis

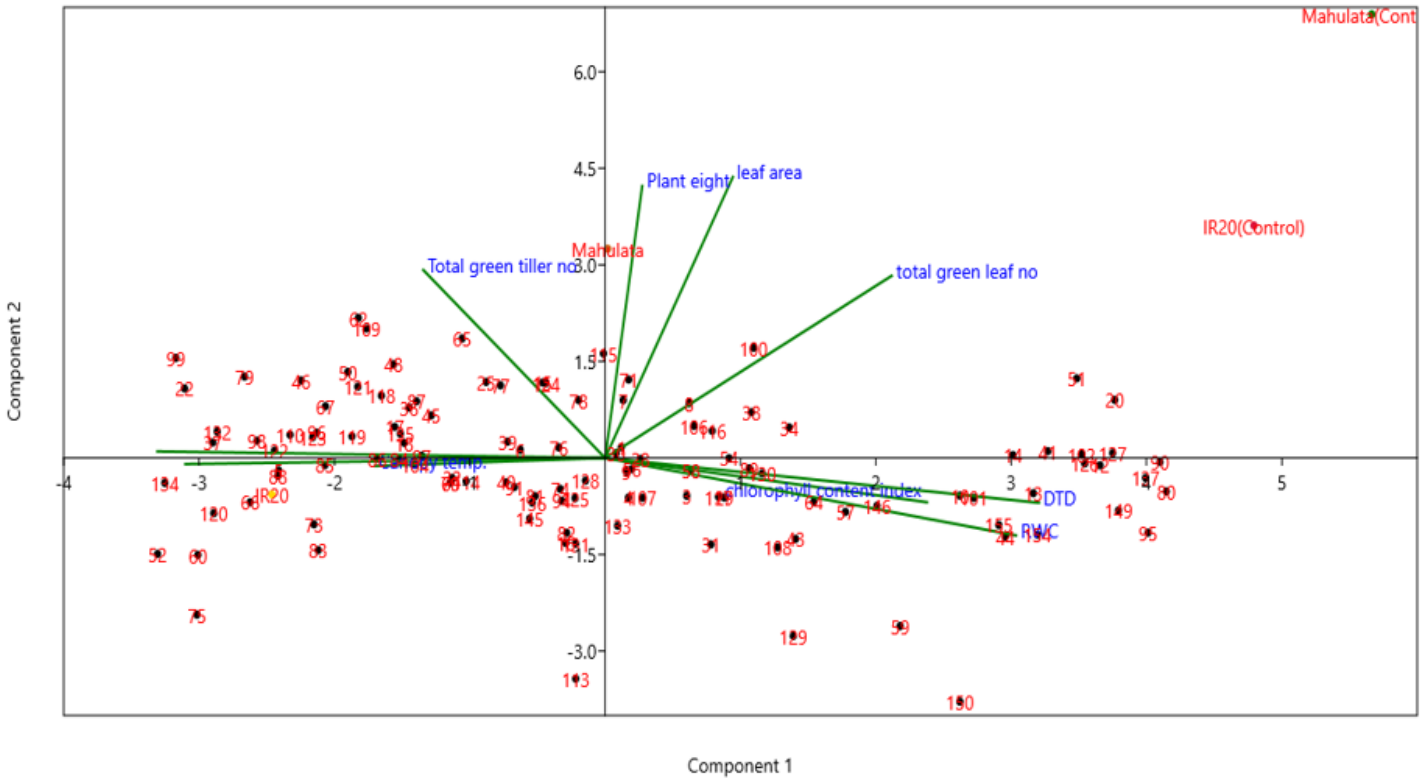

Figure 7

Bi-plot representing the degree and direction of association between variables

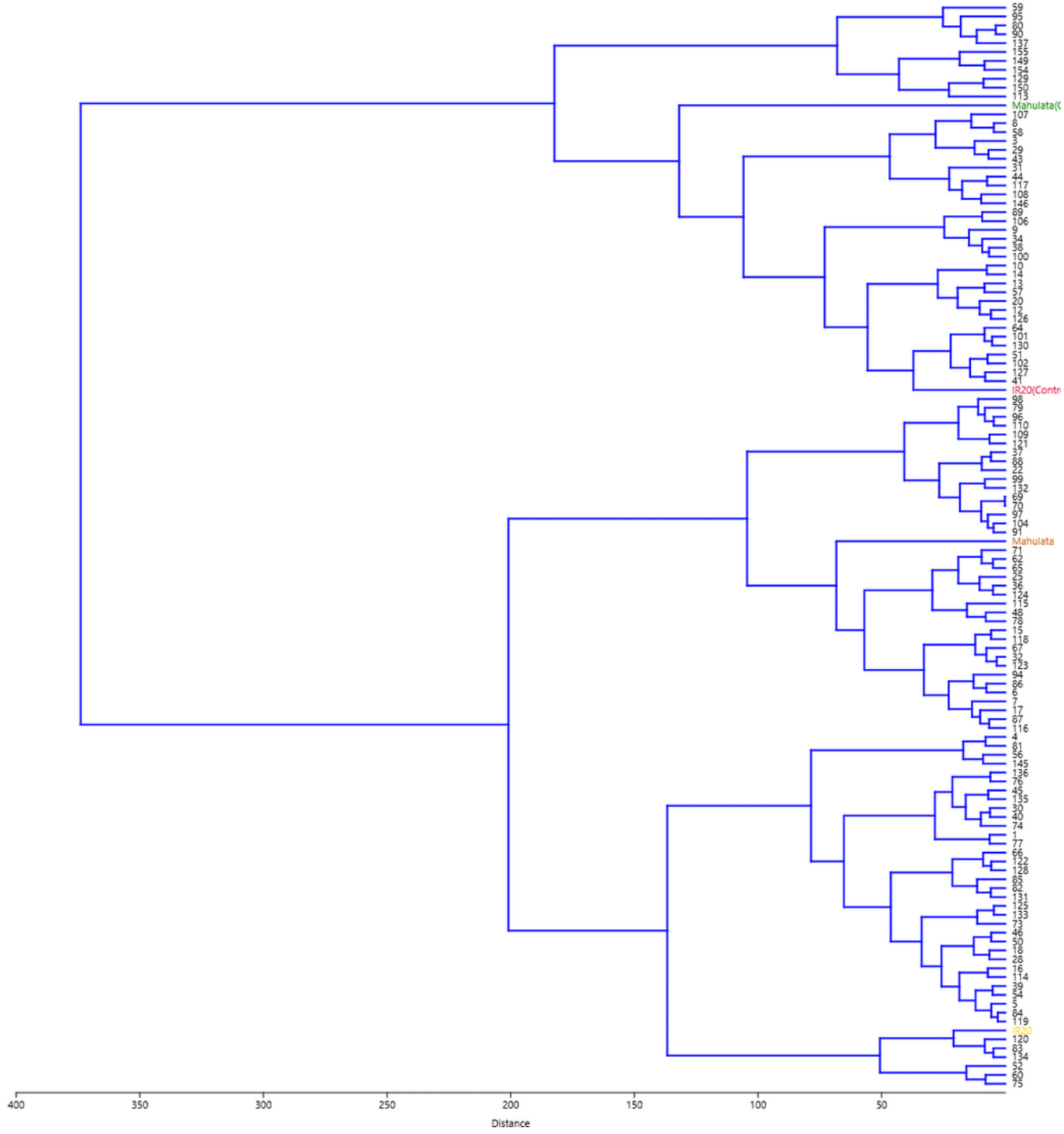

Figure 8

Hierarchical clustering of total population under observation