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Studies on grouping of rice genotypes based on chemical and biochemical tests and the inter-relationship between seed and seedling characters

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Abstract
Rice (*Oryza sativa* L.) is a popular cereal crop commonly used as human food. It is actually a type of grass and belongs to a family of plants Poaceae that includes other cereals such as wheat and corn. Rice is rich in nutrients and contains a number of vitamins and minerals. It is an excellent source of complex carbohydrates - the best source of energy. Rice has a renowned relationship with the humans since ages. Presently, more than half of the world's population depends on rice as a staple food (Barah and Pandey, 2005). For developing laboratory keys for genotype identification, chemical and biochemical tests play an important role. In this study for chemical (Phenol and Modified phenol tests) and biochemical (Alpha-amylase and dehydrogenase enzyme activity) characterization, twenty rice genotypes (PR-124, IABT-17, RP-Bio-226, DRR Dhan-44, Gangavati emergency, GNV-1405, GNV-14-96-1, GNV-1415, GNV-1109, GNV-10-89, IET-23304, IET-26286, IET-24796, KRGL-20, IET-26232, SMW-09-32, IET-26290, IET-255051, MSB-43-1-2 and IET-25574) were used. These genotypes were grouped into five different colour groups *viz.,* light brown, dark brown, black and no colour change. Rapid identification techniques are the testing procedure that utilizes specific tests to reveal chemical and biochemical differences among the seeds of different genotypes. These tests are found to be quick and accurate and used to identify varieties The chemical and biochemical tests served as a great tool in the identification of genotypes. Also developed seed keys for identification of genotypes. Also inter relationship of 100 rice genotypes between seed characters *viz*., length, width, length/width ratio, 1000 seed weight and seedling characters *viz*., germination percentage, shoot length, root length, seedling length, speed of germination, seedling dry weight were assessed by comparing the observations recorded using correlation studies.

Keywords: Paddy, phenol test, modified phenol test, alpha-amylase, dehydrogenase, seed and seedling characters

Introduction
The genotypes of rice were tested for designated chemical tests *viz.,* Phenol and Modified phenol tests and biochemical tests alpha-amylase and dehydrogenase enzyme activity. Based on the colour reaction and enzyme activity of different genotypes seed keys were developed for distinguishing the rice genotypes. The chemical tests grouped the varieties into four classes based on their reaction with the seed coat and the based on enzyme activity the genotypes were grouped as high, medium and low. These simple, reliable and quick tests can be used for varietal identification. Inter relationship between seed and seedling characteristics was observed in the rice genotypes. It was noticed that seed size is positively correlated with seed vigour, larger seeds tend to produce more vigorous seedlings (Ries and Everson, 1973) [16]. Germination rate and seedling vigour index values increased with the increase of seed size suggesting the selection of larger seeds for good stand establishment in rice (Roy *et al.* 1996) [17]. Seed size is positively correlated with seed vigour, larger seeds tend to produce more vigorous seedlings (Cookson *et al.* 2001) [7]. Large seeds expressed high seedling vigour index than the small size seeds in all the varieties, reported by Nagaraju (2001) [13].

Material and Methods
Grouping of genotypes through chemical tests
Phenol test
Four replications of fifty seeds were pre-soaked in distilled water for 24 hours at 25 ± 1 °C. Then they were transferred on to two layers of Whitman No.1 filter paper saturated with one per cent phenol solution.

The petri dishes were covered and incubated at 25 ± 1 °C and the colour reaction was noted after 24 hours. Based on the development of seed coat colour, the selected genotypes were classified into different categories as light brown, dark brown, black colour and no colour change (Jaiswal and Agarwal, 1995) [10].

Modified phenol test

Four replications of fifty seeds were pre-soaked in 0.5 per cent copper sulphate solution for 24 hours at 25 ± 1 °C. Then they were transferred on to two layers of Whitman No.1 filter paper saturated with one per cent phenol solution. The petri plates were covered and incubated at 25 ± 1 °C and the colour reaction was noted after 24 hours of incubation and the genotypes were classified into different categories as light brown, dark brown, black colour and no change colour (Jaiswal and Agarwal, 1995) [10].

Grouping of genotypes by using biochemical test

Dehydrogenase enzyme activity (OD value)

Twenty five representative seeds from each treatment in two replications were taken and pre conditioned by soaking in water overnight at room temperature. The dehusked seeds were steeped in 0.25 per cent solution of 2, 3, 5-triphenyl tetrazolium chloride and kept in dark for two hours at 40 ^{0}C for staining. The stained seeds were thoroughly washed with water and then soaked in 10 ml of 2 methoxy ethanol (methyl cello solve) and kept overnight for extracting the red colour formazan. The intensity of red colour was measured using ELICO UV-VIS spectrophotometer (model SC-159) using blue filter at 470 nm wave length and methyl cellosolve was used as a blank. The OD value obtained was reported as dehydrogenase enzyme activity (Kittock and Law, 1968) [11] and were grouped into three categories *viz.*, low, medium and high.

Alpha amylase activity (mm)

The α-amylase activity was analysed as per the method suggested by Simpson and Naylor (1962) [19]. Two gram of agar shreds and one gram of potato starch was mixed together in water to form paste and the volume was made up to 100 ml with distilled water. The homogenous solution of agar-starch mixture after boiling was poured into sterilized petri plates and allowed to settle in the form of gel after cooling. The pre-soaked (for 8 hours) and half cut seeds (with their half endosperm and embryo portion intact) were placed in the petri plates in such a way that the endospermic part remained in contact with agar-starch gel. The petri plates were closed and kept in dark at 30 ^{0}C. After 48 hours, the petri plates were uniformly smeared with potassium iodide solution (0.44 g of iodine crystal + 20.008 g potassium iodide in 500 ml distilled water) and excess solution was drained off after few minutes. The diameter of halo (clear) zone formed around the seed was measured in mm and reported as alpha amylase activity and were grouped into three categories *viz.*, low, medium and high.

To study the inter relation between seed and seedling characters of rice genotypes.

The inter relationship of 100 rice genotypes between seed characters *viz*., seed length, width, length/width ratio, 1000 seed weight and seedling characters *viz*., germination percentage, shoot length, root length, seedling length, speed of germination, seedling dry weight were assessed by comparing the observations recorded using correlation studies.

Results and Discussion

Results grouping of genotypes based on chemical test

The experimental results on chemical tests *viz.*, phenol and modified phenol tests are presented in table 1.

Table 1: Grouping of genotypes based on chemical tests

Genotypes	Phenol test	Modified phenol test
PR-124	Black	Dark brown
IABT-17	Black	Black
RP-Bio-226	Black	Dark brown
DRR Dhan-44	Dark brown	Dark brown
Gangavati emergency	Dark brown	Black
GNV-1405	Light brown	No colour change
GNV-14-96-1	Light brown	No colour change
GNV-1415	Light brown	Light brown
GNV-1109	No colour change	Light brown
GNV-10-89	No colour change	Light brown
IET-23304	Light brown	Dark brown
IET-26286	Light brown	Light brown
IET-24796	No colour change	No colour change
KRGL-20	Dark brown	Dark brown
IET-26232	Light brown	Black
SMW-09-32	Dark brown	Dark brown
IET-26290	Light brown	No colour
IET-255051	No colour change	Light brown
MSB-43-1-2	Dark brown	Dark brown
IET-25574	Dark Brown	Black

Phenol test

Varied response of rice genotypes to phenol test was observed. Based on the colour development, genotypes were grouped into four group's *viz.*, light brown, dark brown, black and no colour change.

Among the twenty genotypes, seven genotypes showed light brown, six genotypes showed dark brown, three genotypes showed black colour and four genotypes showed no colour change.

Modified phenol test

The rice genotypes used for the modified phenol test sowed varied response. Based on the colour development, genotypes

were grouped into four groups *viz.*, light brown, dark brown, black and no colour change.

Among the twenty genotypes, five genotypes showed light brown, seven showed dark brown, four genotypes showed black colour and four genotypes showed no colour change.

Grouping of genotypes based on biochemical tests

The experimental data on biochemical tests *viz.*, dehydrogenase enzyme activity (OD values at 470 nm) and α-amylase activity (mm) are presented in table 2.

Table 2: Grouping of genotypes based on biochemical tests

Genotypes	Alpha-amylase activity (mm)	Category	Dehydrogenase activity (OD value)	Category
PR-124	11.69	High	0.703	High
IABT-17	10.77	High	0.719	High
RP-Bio-226	9.30	High	0.705	High
DRR Dhan-44	9.71	High	0.716	High
Gangavati emergency	11.91	High	0.725	High
GNV-1405	10.35	High	0.713	High
GNV-14-96-1	10.54	High	0.707	High
GNV-1415	11.62	High	0.710	High
GNV-1109	8.52	Medium	0.698	Medium
GNV-10-89	7.35	Medium	0.692	Medium
IET-23304	8.93	Medium	0.690	Medium
IET-26286	8.67	Medium	0.693	Medium
IET-24796	8.61	Medium	0.697	Medium
KRGL-20	6.54	Low	0.696	Low
IET-26232	6.21	Low	0.544	Low
SMW-09-32	6.35	Low	0.526	Low
IET-26290	6.64	Low	0.582	Low
IET-255051	6.05	Low	0.514	Low
MSB-43-1-2	6.46	Low	0.594	Low
Mean	8.74		0.664	
Min	6.05		0.514	
Max	11.91		0.719	
SEm±	0.09		0.127	
CD@1%	0.34		0.495	

Category: Alpha amylase activity (mm) Category: Dehydrogenase enzyme activity
Low: < 7mm Low: < 0.69
Medium: 7-10mm Medium: 0.69-0.70
High: > 10mm High: > 0.70

Dehydrogenase enzyme activity (OD value)

The dehydrogenase enzyme activity of rice genotypes varied significantly. The mean OD value observed among the genotypes was 0.664. The significantly higher OD value was recorded in Gangavati emergency (0.725) and lowest in IET-255051 (0.514). Among twenty genotypes, six genotypes were categorized into low, five genotypes were categorized into medium and eight genotypes were categorized into high category of dehydrogenase activity.

Based on the variation in the dehydrogenase enzyme activity, the genotypes were grouped as low (< 0.69), medium (0.69-0.70) and high (> 0.70) type of category.

Alpha-amylase activity (mm)

The α-amylase activity varied significantly among the rice genotypes. The mean α-amylase activity among the genotypes was 8.74 mm and it ranged from 6.05 mm to 11.91 mm. The significantly highest α-amylase activity was noticed in Gangavati emergency (11.91 mm) and lowest in IET-255051 (6.05 mm). Among twenty genotypes, six genotypes were categorized into low, five genotypes were categorized into medium and eight genotypes were categorized into high category of alpha amylase activity.

Based on the variation in the alpha amylase activity, the genotypes were grouped as low (< 7 mm), medium (7-9 mm) and high (> 9 mm) type of category.

To study the inter-relationship between seed and seedling characters.

Correlation studies between seed and seedling characters were carried out and observed that seed breadth is positively correlated with seed length (0.538), seed length to breadth ratio is positively correlated with seed length (0.056) and negatively correlated with seed breadth (-0.437). Thousand seed weight is significantly related with seed length (0.616), positively related to seed breadth (0.189) and seed length to breadth ratio (0.167).

The relation of shoot length was observed to be positively significant with seed length (0.337) and thousand seed weight (0.371), positively correlated with seed breadth (0.142) and seed length to breadth ratio (0.128). The root length was positively associated with seed length to breadth ratio (0.038) and significant positively associated with seed length, seed breadth, seedling length and thousand seed weight (0.596), (0.319), (0.704) and (0.493) respectively.

The association of seedling length was found to be positively significant with seed length (0.525), seed breadth (0.262), and thousand seed weight (0.478), shoot length (0.901) and root length (0.942), whereas positively correlated with seed length to breadth ratio (0.083).

The relationship of germination percentage is positively related with seed breadth (0.150), seed length to breadth ratio (0.125), shoot length (0.194) and significant positive association was observed with seed length (0.386), root length (0.290) and seedling length (0.270).

Seedling dry weight was found to be significantly related with seed length, root length, seedling length, (0.443), (0.294), (0.280), positively related with seed breadth (0.190), seed length to breadth ratio (0.158) and germination percentage (0.141), highly significant with thousand seed weight (0.496) and shoot length (0.208).

Seedling vigour index I was positively significant with seed length (0.581), seed breadth (0.271), thousand seed weight (0.505), shoot length (0.831), root length (0.891), seedling length (0.937), germination percentage (0.589), seedling dry weight (0.290) and positively related with seed length to breadth ratio (0.116).

The association of seedling vigour index II was found to be positively significant with seed length (0.482), thousand seed weight (0.523), root length (0.322), seedling length (0.309), germination percentage (0.270), seedling dry weight (0.991), seedling vigour index I (0.362), whereas highly significant association with shoot length (0.233) and seed breadth (0.203). But positive association was observed with seed length to breadth ratio (0.174).

Speed of germination was highly significant related with seed length (0.205), seed length to breadth ratio (0.215), positively associated with seed breadth (0.062), shoot length (0.093), root length (0.165), seedling length (0.148), germination percentage (0.019), seedling vigour index I (0.129), whereas positively significant relationship was with thousand seed weight (0.229), seedling dry weight (0.264) and seedling vigour index II (0.266).

Significant correlation of thousand seed weight (0.616), shoot length (0.337), root length (0.590), seedling length (0.525), germination percentage (0.386), seedling dry weight (0.443), seedling vigour index I (0.581), seedling vigour index II (0.482) and highly significant correlation of speed of germination (0.205) was observed with seed length.

Thousand seed weight was significantly correlated with shoot length (0.371), root length (0.493), seedling length (0.478), germination percentage (0.277), seedling vigour index I (0.505), seedling vigour II (0.523), speed of germination (0.229) and highly significant with seedling dry weight (0.496).

Highly significant correlation of seedling dry weight (0.208) and seedling vigour index II (0.233) was noticed with shoot length and significant correlation was observed with root length (0.704), seedling length (0.901) and seedling vigour index I (0.831).

Root length was significantly correlated with seedling length (0.942), germination percentage (0.290), seedling dry weight (0.294), seedling vigour index I (0.891) and seedling vigour index II (0.322) and positively correlated with speed of germination (0.093).

Seedling length was significantly correlated with germination percentage (0.270), seedling dry weight (0.280) and seedling vigour index I (0.891), highly significant with seedling vigour index II (0.233) and positively related with speed of germination (0.165), which is represented in table 3.

Table 3: The inter-relationship between seed and seedling characters

Column1	1	2	3	4	5	6	7	8	9	10	11	12
1	1											
2	0.538	1										
3	0.056	-0.437	1									
4	0.616*	0.189	0.167	1								
5	0.337*	0.142	0.128	0.371*	1							
6	0.596*	0.319*	0.038	0.493*	0.704*	1						
7	0.525*	0.262*	0.083	0.478*	0.901*	0.942*	1					
8	0.386*	0.150	0.125	0.277*	0.194	0.290*	0.270*	1				
9	0.443*	0.190	0.158	0.496**	0.208**	0.294*	0.280*	0.141	1			
10	0.581*	0.271*	0.116	0.505*	0.831*	0.891*	0.937*	0.589*	0.290*	1		
11	0.482*	0.203**	0.174	0.523*	0.233**	0.322*	0.309*	0.270*	0.991*	0.362*	1	
12	0.205**	0.062	0.215**	0.229*	0.093	0.165	0.148	0.019	0.264*	0.129	0.266*	1

*- Significant at 1% (0.255), **- Significant at 5% (0.196)

1. Seed length 2.Seed breadth 3. Seed length/breadth ratio 4. 1000 seed weight 5. Shoot length 6. Root length 7. Seedling length 8. Germination per cent 9. Seedling dry weight 10. Seedling vigour index 11. Seedling vigour index II 12. Speed of germination

Discussion

For developing laboratory keys for genotype identification, chemical and biochemical tests play an important role. Rapid identification techniques are the testing procedure that utilizes specific tests to reveal chemical and biochemical differences among the seeds of different genotypes. These tests are found to be quick and accurate and used to identify varieties.

Grouping of genotypes based on chemical tests

Various chemical tests are being used to reveal chemical differences among the seeds or seedlings of different genotypes. Varietal identification by morphological characters is laborious, time consuming, tedious and cumbersome. A number of chemical tests have been developed for varietal identification such as phenol test and modified phenol test. These chemical tests are very quick, easy and reproducible (Agrawal and Sharma, 1989).

Phenol test

All the varieties involved in the present study were distinguished from each other. Among the twenty rice genotypes, based on the colour development on the seeds, seven genotypes were having light brown, six genotypes were dark brown, three genotypes showed black colour and four genotypes showed no colour variation. This similar classification of varieties was earlier given by Satisha *et al.* (2001) in sunflower. The difference in colour reaction of seeds seems to be due to differences in genetic back ground

concerning the enzyme system (Chakrabarthy and Agrawal, 1990) in black gram.

The phenol colour test, which is an index of polyphenol oxidase activity, is a simple method for grouping the rice varieties (Oka, 1958; Abrol and Uprety, 1972 and Vanangamudi *et al*. 1988) [14, 1, 21]. During phenol colour test, phenol gets oxidized into dark colour melanin catalysed primarily by the enzyme tyrosine in the seed coat and is under simple genetic control (Bowel *et al.* 1969) [6].

Modified phenol test

All the genotypes responded positively to the modified phenol test with $CuSO_4$ and they were grouped into four groups *viz*., no colour change, light brown, dark brown and black, based on the colour development on the seeds. Among the twenty genotypes, the response of four genotypes were no colour change, five genotypes were light brown, seven genotypes were dark brown in colour and four genotypes were black in colour.

Hence, based on the colour reaction with phenol and modified phenol tests the genotypes can be classified into different groups and the standard phenol test with $CuSO_4$ was found to be better in distinguishing the genotypes. The presence of metallic ions Cu++ in modified test enhances the phenol colour reaction since it is an enzymatic reaction and these ions act as catalyst (Banerjee and Chandra, 1977) [5] which was further, confirmed by Gupta and Agarwal (1988) [9]; Agarwal and Karki (1989) [2]. ‘

Grouping of genotypes based on biochemical tests

Biochemical assays like α-amylase and dehydrogenase enzyme activity are of much helpful in characterizing the genotypes.

Significant variations were also observed among the genotypes with respect to dehydrogenase enzyme activity. The highest dehydrogenase enzyme activity was observed in Gangavati emergency (0.71 nm) and lowest in IET-255051 (0.69 nm) with the mean of 0.70 and the genotypes were classified into three categories low, medium and high. Dehydrogenase enzyme which exists in mitochondria, is necessary for respiratory process indicate the level of seed viability and vigour (Anon., 2012). Increased dehydrogenase activity was due to an index of increased cellular biosynthetic activities like DNA and RNA synthesis that in turn indicate the higher protein and energy production necessary for germination and seedling emergence. Similar studies were carried out by Osborne *et al.* (1980) [15].

The α-amylase activity also varied significantly among the rice genotypes. The highest α-amylase activity was recorded in Gangavati emergency (11.91 mm) and lowest in IET-255051 (7.05 mm) with mean 9.57 mm and the genotypes were classified into three categories low, medium and high. The variation might be due to genetic makeup of the genotypes. The alpha amylase is, increased enzymatic activity due to the chemicals involved in metabolic activity which stimulates the synthesis of enzymes for reserve food mobilization in seeds. Similar studies were reported by Vipul *et al.* (2014) [22], Ganesh *et al.* (2013) [8], Mewael *et al.* (2010) [18] and Trivedi and Rathi (2016) [20].

Inter relationship between seed and seedling characters of paddy genotypes

It is important to know the nature of inter relationship revealing between seed and seedling characters. This can be obtained from simple correlation coefficient which helps a breeder in determining the direction and number of characters to be considered in improving seed and seedling characteristics. In the present investigation, correlation coefficient was worked out for seed and seedling characters.

It was observed that seed breadth is positively associated with seed length (0.538) and seed length to breadth ratio is positively correlated with seed length (0.056) and negatively correlated with seed breadth (-0.437). Thousand seed weight is significant and positively related with seed length (0.616), positively related to seed breadth (0.189) and seed length to breadth ratio (0.167).The relation of shoot length was observed to be positively significant with seed length (0.337) and thousand seed weight (0.371), positively correlated with seed breadth (0.142) and seed length to breadth ratio (0.128). The root length was positively associated with seed length/breadth ratio (0.038), significant and positively associated with seed length, seed breadth, seedling length and thousand seed weight (0.596), (0.319), (0.704) and (0.493) respectively. The association of seedling length was found to be positively significant with seed length (0.525), seed breadth (0.262), thousand seed weight (0.478), shoot length (0.901) and root length (0.942), whereas positively correlated with seed length to breadth ratio (0.083).The relationship of germination percentage is positively related with seed breadth (0.150), seed length to breadth ratio (0.125), shoot length (0.194) and significant positive association was observed with seed length (0.386), root length (0.290) and seedling length (0.270).Seedling dry weight was found positive and significantly related with seed length, root length, seedling length, (0.443), (0.294), (0.280), positively related with seed breadth (0.190), seed length to breadth ratio (0.158) and germination percentage (0.141), highly significant with thousand seed weight (0.496) and shoot length (0.208).Seedling vigour index I was positively significant with seed length (0.581), seed breadth (0.271), thousand seed weight (0.505), shoot length (0.831), root length (0.891), seedling length (0.937), germination percentage (0.589), seedling dry weight (0.290) and positively related with seed length to breadth ratio (0.116).The association of seedling vigour index II was found to be positively significant with seed length (0.482), thousand seed weight (0.523), root length (0.322), seedling length (0.309), germination percentage (0.270), seedling dry weight (0.991), seedling vigour index I (0.362), whereas highly significant association with shoot length (0.233) and seed breadth (0.203). But positive association was observed with seed length to breadth ratio (0.174).Speed of germination was highly significant related with seed length (0.205), seed length to breadth ratio (0.215), positively associated with seed breadth (0.062), shoot length (0.093), root length (0.165), seedling length (0.148), germination percentage (0.019), seedling vigour index-1 (0.129), whereas positively significant relationship was with thousand seed weight (0.229), seedling dry weight (0.264) and seedling vigour index II (0.266), speed of germination is highly significantly correlated (0.215), similar studies were reported by Hussain *et al*. (2010).

Summary and Conclusion

Chemical tests

The seeds were subjected to phenol test for differentiating the genotypes. Based on the colour of the seed, the genotypes were grouped as light brown (seven genotypes), dark brown (six genotypes), black (three genotype) and no colour (four genotypes).

And in modified phenol test, the genotypes were grouped as light brown (five genotypes), dark brown (seven genotypes), black (four genotypes) and no colour (four genotypes).

Biochemical tests

The genotypes recorded variation among the rice genotypes with respect to dehydrogenase activity which ranged from 0.69nm (IET-255051) to 0.71 nm (Gangavathi emergency). The alpha amylase activity recorded variation from 7.05mm (IET-255051) to 11.91mm (Gangavati emergency).

Correlation between seed and seedling characteristics

Both seed and seedling characteristics play an important role in characterization of a genotype. In the present study, except for seed length/breadth ratio was negatively correlated with seed breadth, all other seed and seedling characteristics like seed length, seed breadth, seed length to breadth ratio, thousand seed weight, shoot length, root length, seedling length, seedling dry weight, germination percentage, seedling vigour index I, seedling vigour II and speed of germination are positively related among themselves.

Light brown Brown Dark brown

Black No colour change

Plate 1: Colour change reaction of paddy genotypes in phenol and modified phenol tests.

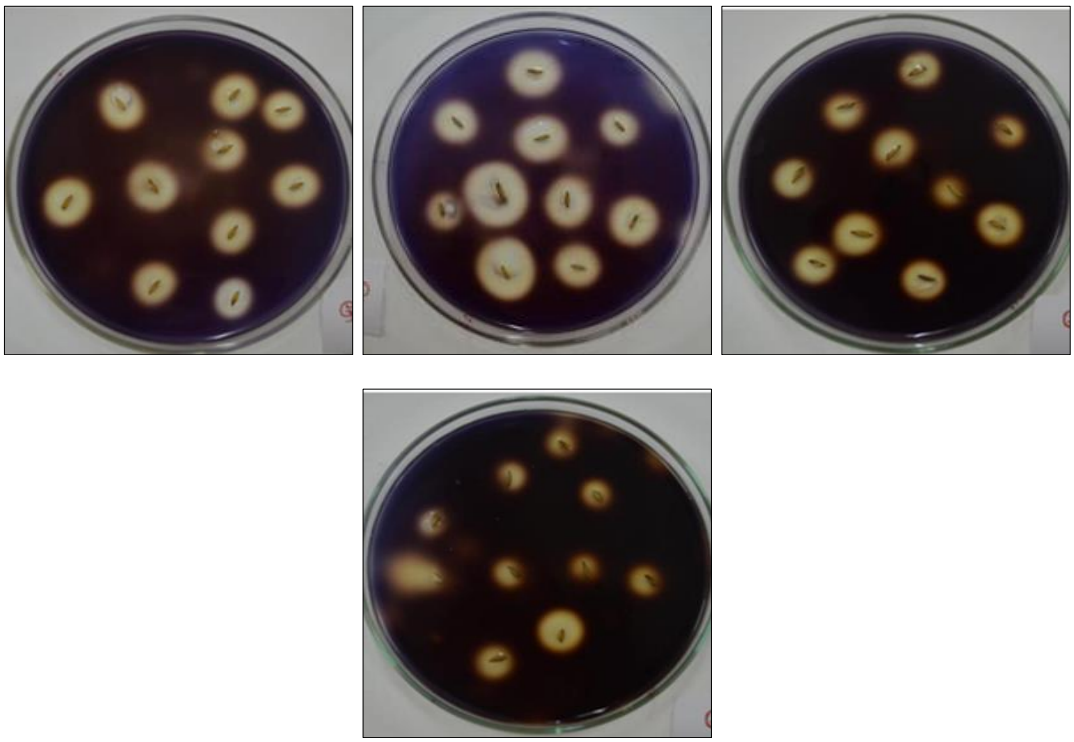

Plate 2: Alpha- amylase activity in paddy genotypes

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