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Role of Seed Treatment with Herbal Plant Products and Management Practices with Growth Nutrients by Decreasing Chlorophyll Reduction Rate for Overcoming Submergence Stress in Rice (*Oryza Sativa* L.)

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Abstract

The fresh harvested dried seeds with optimum moisture content of two paddy genotypes viz. Mahananda and Swarna were treated with sun drying powdered form of nineteen herbal treatments at the rate of 2 gm of treatment/kg of seeds along with control and stored for 3 months at -20°c. The herbal treated seeds were raised to establish as 21 days old seedlings in a pot with soil which were submerged for ten days in the drum in three replications. The same set of herbal treated seeds were sown at low land field condition at Baruipur, South 24 Parganas, West Bengal, India to see the effect of laboratory experiment for two consecutive years 2012 and 2013 in the kharif season and various agro morphological characters are estimated. Under severe submergence stress out of 20 treatments (19 herbals and one control) four treatment like tobacco, kalmegh, neem and vitex leaf have significantly reduce the reduction% of chlorophyll in both the varieties. The chlorophyll reduction% is negatively correlated with survival percentage. The field experiment result reveals that tobacco, kalmegh, neem and vitex leaf treated seeds of the two paddy genotypes have outstanding seed yield. In a separate set up 21 days old rice seedlings of above rice genotypes were treated with growth nutrients viz. nitrogen(N), phosphorous(P), potassium(K) and NPK separately along with control in the soil filled pot prior to two days of submergence for complete ten days in three replications in the drum. Total chlorophyll estimation was done before and after submergence in both lab experiments. Survival % was done after submergence. The total chlorophyll reduction% was higher in case of nitrogen and NPK treatment in both the varieties. Nitrogen has the ill effects over the survival% under submergence condition. Better phosphorous soil status before submergence will enhance the survival% even in the susceptible variety Swarna. Proper and suitable management practices are very crucial for overcoming submergence stress in rice.

Keywords: Submergence, herbal treatments, growth nutrients, chlorophyll reduction%, survival%, agro morphological characters.

Introduction

Rice is the main staple food of India. Rice production in India is utmost priority for economy, livelihood and religious purposes[1]. Rice is cosmopolitan crops which can be grown under various climatic conditions. In South- East Asian countries rice provides above 50% per capita dietary energy and protein, 17-27% of dietary fat[2]. The world population is predicted to touch more or less 800 crore by 2030. There is an utmost need to increase the rice production by 40% without harming the existing ecosystems[3]. Over growth of human population, high demand for land and food, impose new challenges toward food security. Rice is subjected to important biotic and abiotic stresses which dramatically reduce yield globally, with submergence being one of the major abiotic stresses. Abiotic stress is the prime hurdles to crop production in whole world, decreasing average yields of most of the crops by more than 50%[4-5]. Among these, submergence is the 3rd most severe ones[6]. Sustainable agriculture along with proper management practices have an important role to act in conserving natural resources[7]. Herbal plant products can be use as novel system which is less harmful, eco-friendly and alternative option to counteract the effect of chemical growth retardants for increasing seedling vigor, survival % under submergence condition. The effects of herbal treatments are much more cost effective than that of chemical treatments for increasing survivality under submergence condition for paddy genotypes[8]. It was found from some review work, dry and wet treatments with various chemicals and crude plant extracts are effective for overcoming many stresses for several crops[9-12].

Conserving optimum total chlorophyll content after submergence are very crucial to regenerate successfully for rice. Rate of chlorophyll reduction after submergence is inversely proportionate to the survival. So an attempt was made to study the role of herbal plant products for improving survivality by decreasing chlorophyll reduction rate in selected paddy genotypes Mahananda and Swarna. Suitable germplasm and management practices are crucial to increase rice production in the submerged areas[13]. Rapid regeneration preceded by submergence is an ideal trait under flash or long submergence because it will ensures fast recovery with sufficient biomass. Persistent initiation of new leaves requires availability of

optimum chlorophyll concentration after submergence[14-15]. Proper nutrient management may also increase recovery and grain yield after submergence. Addition of phosphorous to the soil leads to increase submergence tolerance of plants. Survival of seedling of various crop genotypes decreased sufficiently with high nitrogen content both in soil and seedlings whereas submergence stress tolerance is negatively correlated with leaf nitrogen content after submergence[16]. Avoiding application of excessive dose of nitrogen may increase the crop production in the low land submerged field areas. Nitrogen has ill effects over the survival % under submergence condition. Better phosphorus soil status before submergence will enhance the survival % even in the susceptible variety. So an attempt was made to see the effect of different growth nutrients viz. nitrogen (N), phosphorous (P), potassium (K) and NPK as integrated management practices for better survival percentage after submergence in rice.

Material and Methods

Management by herbal seed treatments at laboratory condition: Mahananda and Swarna two paddy genotypes were taken for this experimental study. The objective of the study was to give the management practice for submergence tolerance. The harvested fresh seeds of the two genotypes were dried properly to attain the moisture content 8-10 %. Nineteen herbal treatments were used in dust form and the dust were prepared by sun drying the herbal material, and then powdered by mortar and pestle. The list of herbal treatment is given in table-1.

Table-1
List of herbal plant treatments along with their scientific name

Control (No herbal treatment)	Coriander Powder (*Coriandrum sativum*)
Tobacco leaf Powder (Nicotiana tabacum.)	Hing Powder (*Ferula asafoetida*)
Kalmegh leaf Powder (*Andrographis paniculata)*	Jeera Powder (*Cuminum cyminum)*
Neem leaf Powder (*Azadirachta indica*)	Betel vine leaf Powder (Piper betle)
Vitex leaf Powder (*Vitex* negundo)	Bel leaf powder (Aegle marmelos)
Lemon leaf (*Citrus limon*)	Garlic Powder (*Allium sativum*)
Ginger Powder (*Zingiber officinale)*	Tea (Camellia sinensis)
Onion Powder (*Allium cepa*)	Coffee (Coffea arabica)
Turmeric Powder (*Curcuma longa*)	Eucalyptus leaf powder (*Eucalyptus* globules)
Chilli Powder (*Capsicum annum*)	Garam Masala

The treatments were given to the seeds of the rate of 2g of treatment/kg of seeds and these treated seeds were stored at -20^0c for three months before conducting the experiment. The control was devoid of any treatment. After 3 months these treated and controlled seeds were placed in germination plate. Five seeds were taken for germination in 3 replications. After 3 days the germination% were counted and the germinated seeds were transferred to the pot filled with soil. 21 days old seedlings were completely submerged in the drum for ten days. After ten days the survival% were recorded. Compare of treatments effect with control was done for submergence tolerance in rice. Chlorophyll content was estimated before and after submergence with treatment effect along with control.

Statistical Procedure: Survival percentage: {(No. of plants before submergence – No. of dead plants)/No. of plants before submergence} x100

Elongation per day: = (Plant height after submergence-Plant height before submergence)/No. of days submerged.

Elongation% (E %) = {(Plant height after submergence – Plant height before submergence)/ Plant height before submergence.} x100

Survival Scores (SES 1-9): The standard evaluation system of rice was followed for determination of survival score by IRRI[17].

Low land field screening: The field experiment was conducted in Calcutta University Experimental Farm, Baruipur, South 24 Parganas in the aman season of 2012 and 2013 for two consecutive years under low land submerged condition. The aim of this experiment was to study the effects of the herbal seed treatments on yield, and yield related characters in field conditions under submergence stress. The experimental plot was puddle and after final land preparation, farmyard manure (FYM) was given at the rate of 1 ton/hectare. The experimental plot was divided into three blocks and in each block all of the 19 herbal treated seeds and their control were sown in lines. Treated seeds, as mentioned earlier were taken for conducting the experiment. Two pre-treated germinated seed were sown in each pit along the line with a spacing of 20 cm and the spacing between two lines was 30cm. The seeds were sown in Randomized Block Design (RBD) with three replications. Fully grown rice plants were harvested in the month of November for consecutive two years. The varieties were bundled up separately and then allowed to dry. Five plants were taken randomly in each replication and data were taken for plant height, number of tillers/hill, number of panicles/plant, number of grains/panicle, length of the panicle, 1000 seed weight, seed yield/plant and their mean were computed.

Management by treatment with growth nutrients (N, P, K and NPK): Two rice varieties viz. Mahananda and Swarna were chosen for this experiment. 21 days rice seedling treated with nutrients at the rate 2.0gm in the pot filled with soil two days before submergence and were submerged for 10 days. Chlorophyll content was estimated along with survival for nutrient status before submergence. Four growth nutrients viz.

Nitrogen (N), Phosphorous (P), Potassium (K), and NPK were taken along with control for their effect in tolerance. After ten days of submergence their survival percentage were recorded for each treatment along with the estimation of chlorophyll content.

Estimation of chlorophyll content: The leaf chlorophyll was extracted by the slight modification of Hiscox and Israelstam, 1979, by DMSO (Dimethylsulfoxide) method[18]. The chlorophyll data was taken for 21 days seedlings followed by 10 days submergence.

Chlorophyll Estimation by DMSO (Dimethyl Sulfoxide) method: 0.1 gm of dry leaf tissue was taken and cut into small pieces and suspended in graduated test tube containing 2 ml of DMSO (EMPLURA, Merck) with three replications. Test tubes were incubated at 61^0C temperature for 20 minutes in a water bath. The supernatant was decanted and another 3 ml of DMSO was added to the residue and incubated at 61^0C for 20 minutes. The supernatant were pooled and the volume was made up to 10 ml by adding DMSO. The chlorophyll extract was transformed to a cuvette and the absorbance was read in a spectro photometer (Microcontroller Based Visible Scanning Spectro Photometer CL-350, Chemi Line) at 645 and 663 nm against DMSO Blank. Total chlorophyll content along with other components were calculated by the formulae proposed by Arnon,1949[19].

Results and Discussion

It was found from the table- 2 that under severe submergence stress out of 20 treatments (19 herbals and 1 control) four treatments like tobacco, kalmegh, neem and vitex leaf have significantly reduce reduction percentage of chlorophyll in both Mahananda and Swarna. In susceptible variety Swarna, the four treatments effect was significant with Kalmegh showing better performance over other treatments. The chlorophyll reduction percentage is negatively co related with survival percentage. In Mahananda, treatments like tobacco, neem, kalmegh and vitex exhibited 80% survival and 28.6 % chlorophyll reduction, 70% survival and 40% chlorophyll reduction, 70% survival and 44% chlorophyll reduction and 60% survival and 50% chlorophyll reduction respectively along with the control with 50% chlorophyll reduction and 60% survival. In case of Swarna, treatment kalmegh leaf showed best with highest chlorophyll reduction% 40 and 50% survival over the control with 83.3% of chlorophyll reduction and 10% survival.

Table-2
Effect of herbal treatments on chlorophyll content in rice plant during after and before submergence (21 days seedlings + 10 days submergence)

Variety	Mahananda				Swarna			
Herbal treatment	Total chlorophyll(mg/g)		Reduction %	Survival%	Total chlorophyll(mg/g)		Reduction%	Survival %
	Before submergence	After submergence			Before submergence	After submergence		
Control	06	03	50.0	60	06	01	83.3	10
Tobacco	07	05	28.6	80	06	03	50.0	40
Kal megh	05	03	40.0	70	05	03	40.0	50
Neem leaf	05	03	40.0	70	04	02	50.0	40
Vitex leaf	04	02	50.0	60	04	02	50.0	30
Lemon leaf	04	02	50.0	60	03	0.65	78.3	00
Ginger	04	02	50.0	60	02	0.69	65.5	00
Onion	04	02	50.0	50	02	0.38	81.0	00
Turmeric	05	02	60.0	40	03	01	66.7	10
Chilli	06	03	50.0	50	4.5	02	55.6	10
Coriander	05	02	60.0	50	05	02	60.0	20
Hing	05	01	80.0	30	05	02	60.0	10
Jeera	05	02	60.0	40	06	02	66.7	10
Betel vine	06	03	50.0	40	05	01	80.0	10
Bel leaf	05	02	60.0	30	06	02	66.7	00
Garlic	04	01	75.0	30	04	01	75.0	00
Tea	04	02	50.0	40	04	01	75.0	00
Coffee	04	01	75.0	50	05	02	60.0	00
Eucalyptus	05	02	60.0	40	05	02	60.0	10
Garam masala	06	03	50.0	60	06	03	50.0	10

Table-3 reveals the result of effect of nutrients (N, P, and K and NPK) on chlorophyll in rice plant during after and before submergence for 10 days submergence. In Mahananda, the total chlorophyll content was for control, phosphorus, potassium, nitrogen and NPK treatments are 8.7, 9.6, 9.1, 14.9 and 15.5 mg/g respectively before submergence and 3.6, 5.1, 4.3, 2.8 and 2.7 mg/gm respectively after submergence. In Mahananda both phosphorous and potassium have the good effect by reducing the chlorophyll reduction% with the values 46.9 and 52.7% along with better survival with 70 and 60% respectively after submergence. Both nitrogen and NPK have the greater chlorophyll reduction % over 81 % with 20 % survival after submergence in Mahananda. The chlorophyll reduction% was higher in case of nitrogen and NPK treatment in both the varieties. Swarna exhibited better survival with phosphorus treatments. Nitrogen has bad effects over the survival% under submergence condition. Optimum phosphorus soil status before submergence will promote the survival% even in the susceptible variety.

The mean of different agro-morphological characters including yield of Mahananda and Swarna paddy genotype for two consecutive years 2012 and 2013 at harvesting stage was studied to see effect of herbal seed treatment under low land submergence condition with respect to their control which were given in table-4. Highest seed yield was recorded in neem leaf for Mahananda. In Mahananda the treatments like tobacco, kalmegh and vitex leaf exhibited outstanding performances over the control.

Highest seed yield was observed in tobacco leaf for Swarna, where as kalmegh, neem leaf, turmeric, hing and garam masala gave better seed yield over the control. Tobacco, neem and kalmegh gave better performances in both the varieties.

Conclusion

The study of effect of herbal plant products and proper nutrient management practices are a holistic approach for improving seedling vigor along with survival percentages under submergence stress in rice. By decreasing the chlorophyll reduction% after the submergence will be the one of the most important criteria for developing submergence tolerant rice varieties. The status of this kind of experiment is very important for the improvement of agricultural productivity through sustainable and integrated agriculture.

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Table-3
Effect of nutrients (N, P, K and NPK) on chlorophyll in rice plant during after and before submergence (21 days seedlings + 10 days submergence)

Variety	Mahananda				Swarna			
Nutrient treatments	Total chlorophyll(mg/g)		Reduction %	Survival %	Total chlorophyll (mg/g)		Reduction %	Survival %
	Before submergence	After submergence			Before submergence	After submergence		
Control	8.7	3.6	58.6	60	7.6	2.5	67.1	10
Phosphorus (SSP)	9.6	5.1	46.9	70	10.5	4.3	59.0	30
Potassium (MOP)	9.1	4.3	52.7	60	9.2	2.3	75.0	10
Nitrogen (UREA)	14.9	2.8	81.2	20	10.6	0.8	92.5	0.33
N:P:K (10:26:26)	15.5	2.7	82.6	20	13.7	1.1	92.0	0.33

Table-4
Mean values of various agro morphological characters for submergence tolerance in rice under low land condition at Baruipur for herbal treated paddy seeds for two consecutive years 2012 and 2013

Variety	Treatments	Plant height (cm)	Tillers/ hill	Panicles/ hill	Grains/panicle	Panicle length (cm)	1000 seed wt(g)	Seed yield/ plant (g)
Mahananda	Control	140.1	21.1	19.6	186.3	27.5	30.1	30.1
	Tobacco	142.3	28.1	24.1	213.1	28.1	32.2	76.3
	Kalmegh	145.2	32.2	26.3	210.8	27.6	35.1	64.3
	Neem leaf	140.3	30.3	23.3	221.3	26.3	35.1	89.5
	Vitex leaf	150.1	27.1	22.3	200.5	26.2	32.3	41.2
	Lemon leaf	146.3	24.2	17.2	155.6	27.5	28.1	27.3
	Ginger	142.3	20.2	14.1	157.3	26.1	29.3	27.2
	Onion	145.1	18.3	15.2	161.3	26.5	31.2	28.1
	Turmeric	140.1	19.1	13.3	155.1	27.3	30.5	26.3
	Chilli	142.2	14.2	13.3	177.3	27.2	30.3	29.5
	Coriander	146.2	13.1	11.3	162.3	28.3	32.1	28.1
	Hing	150.2	12.1	10.1	160.1	26.5	31.2	27.3
	Jeera	151.3	13.3	12.3	152.1	27.5	28.5	26.1
	Betel vine	144.3	15.2	13.1	147.1	26.1	28.5	25.3
	Bel leaf	146.1	11.3	10.2	143.3	27.3	29.5	24.2
	Garlic	147.1	14.1	12.2	151.3	27.5	30.1	26.1
	Tea	151.2	12.2	10.3	117.8	26.5	32.3	23.5
	Coffee	153.3	12.1	10.5	113.1	26.5	31.3	22.3
	Eucalyptus	152.3	14.2	13.6	124.1	27.3	29.5	24.1
	Garam masala	150.3	17.1	16.1	154.1	27.5	30.2	26.3
CD		1.45	1.71	3.1	1.62	1.31	1.73	1.52
Swarna	Control	85.1	12.2	11.1	103.1	26.5	20.3	15.1
	Tobacco	84.1	14.3	13.2	147.6	27.3	24.3	25.3
	Kalmegh	75.2	12.3	11.3	130.3	27.1	22.1	24.3
	Neem leaf	90.3	13.3	12.3	112.3	28.5	25.3	21.3
	Vitex leaf	90.5	10.1	9.3	102.3	27.5	23.3	15.3
	Lemon leaf	76.3	9.1	8.1	99.1	26.3	20.5	12.1
	Ginger	75.1	10.2	9.1	100.3	28.1	18.8	14.3
	Onion	87.1	10.5	9.2	100.1	26.3	20.5	13.3
	Turmeric	89.3	11.3	11.3	105.3	26.5	19.3	16.1
	Chilli	93.2	9.6	8.3	104.1	27.5	21.3	15.1
	Coriander	87.3	9.1	9.3	93.1	28.1	18.5	14.1
	Hing	91.1	11.2	10.1	110.1	28.5	18.1	17.2
	Jeera	89.1	10.3	9.3	97.3	26.3	20.3	13.3
	Betel vine	92.3	8.1	8.3	91.2	26.5	19.3	14.5
	Bel leaf	95.3	10.1	10.3	87.6	27.1	18.7	11.1
	Garlic	88.2	12.3	11.2	91.3	26.5	20.2	12.2
	Tea	94.3	11.3	10.1	87.1	27.5	19.1	10.1
	Coffee	91.3	10.2	9.1	105.3	28.3	18.3	15.3
	Eucalyptus	93.1	10.1	9.3	89.1	26.3	17.3	11.3
	Garam masala	87.5	11.3	10.2	93.3	26.5	18.1	16.1
CD		1.22	1.69	2.60	1.17	1.31	0.95	2.10

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