Original Research Article

Enhancing Planting Value of Rice Seed through Priming with Humic Substance

.**ABSTRACT**

A study was carried out at seed testing laboratory of Institute of Agricultural Research and Training, Obafemi Awolowo University, Ibadan to examine the response of rice to treatment with Plant Growth Regulator (Vimpel^{TM}) developed in Nigeria, with a view to determining the effect of the humic substance on germination and seedling growth rate of the seed. FARO 44 rice variety was divided to five parts before priming each lot with four varied concentrations: (0%, 25%, 50% and 100%) and one control (dry unprimed seed). Fifty seeds in three replicates were drawn from each concentration after two (2), four (4) and eight (8) hours of priming (duration) and planted into separate planting round transparent plastic bowl filled with sterilized river sand. Each treatment was replicated three times and the experiment was repeated twice. Data was collected on final germination percentage (FGP), mean germination time (MGT), coefficient of velocity of germination (CVG), seedling vigour index (SVI), germination rate index (GRI), relative seedling growth rate (RSGR) and speed of germination index (SGI). Rice seed priming with PGR (Vimpel^{TM}) did not significantly improve seed germination but the growth rate is significantly influenced with priming with humic substance. Priming with 50% concentration of humic substance for 4 hours significantly improved the seedling growth rate of rice. Therefore, priming rice seed with humic substance can improve seedling growth and vigour of rice under upland and lowland cultivation.

Keywords: Rice seed, Priming, Humic substance, Seedling growth.

1. INTRODUCTION

Rice (*Oryza sativa*) is the world's most important staple food in Africa and Latin America [1]. Its global production has been estimated to be at the level of' 650 million tones and the area under cultivation have been estimated at 156 million hectares [2]. Rice is known as semi aquatic, annual grass plant and grows in a wide range of soil and water regime: irrigated, rain fed lowland, upland and flood prone. Peasant farmers are the largest producers of food consumed in most developing nations including Nigeria; however, most of these farmers are resource poor and unable to engage in profitable agriculture that can sustain food sufficiency in those countries. These farmers have been adopting various means to boost their agricultural production. Rice requires some important nutrients to grow effectively for higher yield [3]. A common approach to supply the nutrients to the crop is through fertilizer application because of varying levels of soil degradation and fertility. About 70% of the farmers agreed that the use of fertilizer can lead to increased yield [4], however, inaccessibility of farmers to chemicals and fertilizers due to cost and availability has been a major concern to rice farmers. Also, continuous use of chemical fertilizer has been reported to have deleterious effect on soil [5]. It therefore becomes necessary to seek a more sustainable and cost effective approach to supply these nutrients to rice plant. Seed treatment through priming has been identified as an attractive approach to enhance crop establishment [6]. Treatment of' seeds with a variety of inorganic and/or organic compounds, some of which are synthetic, has been successfully demonstrated to improve germination and seedling establishment in seeds of many field crop such as wheat, soybean, sunflower and maize [7]. Also, [8] reported that seed priming of maize, wheat, rice and canola resulted into better germination and establishment. [9] concurred that treatment of seeds with the right products has the potential to improve seedling emergence and establishment as well as plant stands. Humic substances are “end product” of decaying organic matter. They have positive effects on plant physiology by influencing nutrient uptake and root architecture [10]. Humic substances can be likened to bio-stimulants which are non-fertilizer products that have beneficial effect on plant growth. The European Bio-stimulants Industry Council (EBIC) described plant bio-stimulants as substances that contains micro-organisms that stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, when applied to plants or the rhizosphere in small quantities [11]. There are commercial bio-stimulants and inorganic products that are claimed by the manufacturers to play an important role in complementing plant morphological and physiological growth [12].

Since, there is elevated interest in finding alternative measures to manipulate either seed germination or seedling growth or both in an attempt to ensure there is an optimum plant stand with vigorous vegetation growth. This study, therefore examined the seedling growth response of rice to seed priming with humic substance (Vimpel^{TM}) with the objectives of determining the appropriate concentration and period of priming that will enhance germination, seedling vigour and growth rate of rice.

2. MATERIAL AND METHODS

The study was carried out in Seed Testing laboratory of Institute of Agricultural Research and Training, Ibadan, Nigeria. One kg seed of FARO 44 rice variety, sourced from Africa_Rice Centre, Ibadan was first divided into two equal parts. Each halve was then divided into five parts of 100g each, with each part representing varied Vimpel concentrations: 0% (100ml of distilled water only), 25% (25mls Vimpel+75mls of distilled water), 50% (50mls of Vimpel +50mls of distilled water), 100% (100mls of Vimpel only) and Control (Dry unprimed seed). Fifty seeds in three replicates were drawn from each concentration after two (2), four (4) and eight (8) hours of priming (duration) and planted into separate planting round transparent plastic bowl filled with sterilized river sand in three replicates. The river sand was adequately moistened before planting and regularly on daily basis. The bowls were arranged using Completely Randomized Design (RCD) in the seed testing laboratory and allowed to grow under ambient environment of 24^{o}C and relative humidity of 63%. Each planting bowl represented each replicate and the experiment was conducted twice. Data were collected on germinated seedlings as follows:

i. Germination count: Number of seedlings germinating each day was counted on daily basis starting from the date of first emergence (4 days) to 13 days after planting when there was no more germinating seed.

ii. Shoot length: Ten randomly selected seedlings were tagged using paper tape and numbered in each bowl without bias. The length was measured from the base of the shoot to top of the crop on daily basis using transparent ruler and recorded every of other day beginning from 7 days after planting.

Data collected were used to estimate the following seed germination and seedling vigour characters:

i. Final germination percentage (FGP) was determined by finding the ratio of normal germinated seed at 13 days after sowing to total number of seeds planted. This is in according to the method suggested by [13]:

$$FGP = \frac{\text{Number of germinated seedling at final day}}{\text{Number of seed planted}} \times 100$$

ii. Mean Germination Time (MGT): This represents the mean time a seed lot requires to initiate and end germination.

iii. Coefficient of Velocity of Germination (CVG) : This is an estimate of the rapidity of germination of the seed lot and it was estimated according to the method described by Scott *et al*, (1984) :

$$CVG = \frac{\Sigma Ni}{\Sigma NiTi} x100$$

where: N is the number of seeds germinated each day and T is the number of days corresponding to N

iv. Seedling Vigour Index (SVI): It was evaluated on the fourteenth day of planting using the formula of [14] based on the product of germination (%) and seedling length at the 14th day after planting as follows:

$$SVI= \frac{(Germination \% \; x \; Seedling \; length)}{100}$$

v. Germination rate index (GRI): This gives an indication of the percentage of seeds germinating each day of the germination period. It is calculated as

$$GRI = \frac{G1}{1} + \frac{G2}{2} + \frac{G3}{3} + \cdots \ldots \ldots \ldots \frac{Gx}{x}$$

where G1 = Germination percentage x 100 at the first day after sowing,
G2 = Germination percentage x 100 at the second day after sowing

vi. Relative Seedling Growth Rate (RSGR): This is a measure of the increase in seedling growth over a period of time. It is measured as the mass increase in the shoot length above soil level per day from the onset of the measurement till the termination of the shoots and calculated by modifying the equation described by [15] for crop growth rate as

$$RSGR= \frac{(H2-H1)}{(D2-D1)}$$

where H1 = Plant height (cm) recorded at time D1,
H2 = Plant height (cm) recorded at time D2,
D1 and D2 were the interval of days respectively (cm/day)

vii. Speed of germination index (SGI): Speed of germination index was calculated as described by [16]AOSA (2003) :

$$SGI = \frac{\text{Number of germinated seeds at Day 1} + \cdots + \text{Number of germinated seed at the last day}}{\text{Daysof first count at Day 1} + \cdots + \text{days offinal count}}$$

Data obtained were subjected to combined analyses of variance (ANOVA). Difference between the treatments was separated using Duncan Multiple Range Test (DMRT) at 5 % or 1 % levels of significance. Pearson's coefficient of correlations between pairs of seed germination indices and seedling growth characters were determined using [17].

3. RESULTS

Analysis of Variance for Seed Germination Indices and Seedling Growth Characters

Combined analysis of variance (ANOVA) revealed highly significant difference between the experiments (E) for all the seed germination indices: final germination percentage (FGP) and

Comment [H1]: It is necessary to organise the indicators in two categories: germination and related parameters, also seedling growth parameters.

seedling growth parameters: mean germination time (MGT), coefficient of velocity of germination (CVG), seedling vigour index (SVI), germination rate index (GRI), relative seedling growth rate (RSGR) and speed of germination index (SGI)] at *P*< 0.01 (*Table 1*). Also, there were highly significant differences in the response of the rice seed to varied concentration of humic substance (C) used for priming. The duration of priming (D) had high significant effects on all of the characters measured. Interaction of the humic concentrations (C) and duration of priming (D) revealed significant interactive effects on most of the characters measured between the experiments, whereas interaction between the experiments (E) and duration of priming (D) as well as interaction between the experiments (E), concentration of humic substance (C) and duration of priming (D) were not significant for all of the traits studied. The interaction between concentration of humic substance (C) and duration of priming (D) only had significant effects on MGT and RSGR in this study (*Table 1*). Coefficient of variation (CV) ranged from 3.29% for MGT to 21.47% for RSGR.

Table 1: Combined Analysis of variance (ANOVA) for seed germination indices and seedling growth characters of rice as affected by priming with humic substance

SV	df	FGP	MGT	CVG	SVI	SGI	GRI	RSGR
Experiments (E)	1	1472.18**	1.56**	375.03**	224.05**	50884.44**	2653.32**	1.26**
Concentration(C)	4	26.73	0.87**	7.16	22.76**	3465.78**	300.15**	1.71**
Duration (D)	2	659.24**	0.84**	102.08**	37.51**	11764.21**	223.02**	3.67**
E x C	4	35.18	0.80**	6.51	7.25	3902.33**	309.99**	0.31*
E x D	2	32.31	0.04	15.48	0.92	931.01	33.05	0.02
C x D	8	75.30	1.33**	18.24	5.48	1209.54	27.12	0.84**
E x C x D	8	205.64	0.04	37.22	13.44	4413.73	107.06	0.09
Pooled error	60	41.96	0.04	11.16	3.47	849.69	21.74	0.11
Mean		83.96	5.98	18.80	15.15	378.89	55.64	1.53
Minimum		60.00	4.09	9.00	8.47	266.00	37.50	0.10
Maximum		100.00	7.17	30.50	22.42	464.00	78.09	2.80
SE(m)		0.99	0.05	0.47	0.31	5.02	1.00	0.06
CV%		7.72	3.29	17.77	12.30	7.69	8.38	21.47

**, ** Significant at (P = 0.05) and (P =0.01) respectively*

SV: Source of variation; df: degree of freedom; SE(m): Standard error of mean; CV: Coefficient of variation; FGP: Final germination percentage; MGT: Mean germination time; CVG: Coefficient of velocity of germination; SVI: Seedling vigour index; GRI: Germination rate index; RSGR: Relative seedling growth rate; SGI: Speed of germination index

Seed germination indices and seedling growth characters of rice as affected by priming with varied concentrations of humic substance

The final germination percentage (FGT) and coefficient of velocity of germination (CVG) were not significantly affected by the concentrations of the humic substance (*Table 2*). Although, there was no significant difference in the Mean germination time (MGT), Seedling vigour index (SVI) and relative seedling growth rate (RSGR) of rice seed primed with 0%, 25% and 50% concentrations of humic substance, there were corresponding increase in the MGT and SVI as priming concentrations increases, with seed primed with 100% concentration of the humic substance recorded significantly higher MGT (62) and SVI (16.48), when compared with other concentrations and the unprimed seed. Conversely, the germination rate index (GRI) and seedling germination index (SGI) decreases as the priming concentration increases (*Table 2*).

Table 2: Effect of priming rice seed with varied concentration of humic substance on seed germination indices and seedling growth rate of rice seed.

Concentration	FGP	MGT	CVG	SVI	SGI	GRI	RSGR
Control (Un-primed)	85.33a	5.62c	18.54a	13.50c	401.50a	62.45a	1.09c
0%	85.22a	6.06b	19.22a	15.61ab	380.72b	55.19b	1.49b
25%	82.89a	5.98b	17.81a	15.49ab	373.89b	54.97b	1.57b
50%	85.34a	6.02b	19.31a	14.67ab	373.72b	54.15ab	1.51b
100%	83.00a	6.22a	19.14a	16.48a	364.61b	51.46c	1.96a

Mean followed with same alphabet along the same column are not significantly different from each other at 5% significant level

Seed germination indices and seedling growth characters of rice as affected by duration of priming with humic substance

The FGP, SVI, SGI and GRI of rice seed primed with humic substance for four and eight hours were not significantly different from each other. However, rice seeds primed for 8 hours were consistently recorded higher value for FGP, SVI, SGI, GRI and RSGR. All the seed germination indices and seedling growth except CVG increased as duration of priming increases from 2 to 4 hours. (*Table3*).

Table 3: Seed germination indices and seedling growth rate of rice seed as affected by duration of priming with humic substance

Duration	FGP	MGT	CVG	SVI	SG1	GRI	RSGR
2 hours	78.73b	5.83c	16.94c	13.92b	356.70b	52.50b	1.40b
4 hours	85.33a	5.95b	20.63a	15.43a	385.20a	57.09a	1.25b
8 hours	87.80a	6.16a	18.84b	16.10a	394.77a	57.34a	1.92a

Means with the same letter(s) in the same column or row are not significantly different from each other at P = 0.05

FGP: final germination percentage; MGT: mean germination time; CVG: coefficient of velocity of germination; SVI: seedling vigour index; GRI: germination rate index; RSGR: relative seedling growth rate; SGI: speed of germination index

Variation of seed germination indices and seedling growth characters measured in the two experiments

The mean of seed germination indices and seedling growth characters across the treatments (duration of priming and concentration regimes) shows that Experiment 1 had higher overall mean values than Experiment 2 for all of the characters measured in this study except MGT (*Table 4*).

Table 4: Seed germination indices and seedling growth parameters measured as affected by frequency of experiments

Traits	Experiment	Mean	Std. Error
Final germination percentage	1	88.00a	0.966
	2	79.91b	0.966
Mean germination time	1	5.851b	0.029
	2	6.11a	0.029
Coefficient of velocity of germination	1	20.84a	0.498
	2	16.76b	0.498
Seedling vigour index	1	16.72a	0.278
	2	13.57b	0.278
Speed of germination index	1	402.67a	4.345
	2	355.11b	4.345
Germination rate index	1	61.07a	0.695
	2	50.22b	0.695
Relative seedling growth rate	1	1.644a	0.049
	2	1.408b	0.049

Means with the same letter(s) in the same column or row are not significantly different from each other at P = 0.05

Pearson Correlation between pairs of seed germination indices and seedling growth characters

The Pearson correlation coefficient between pairs of seed germination indices and seedling growth characters across the experiments are presented in Table 5. FGP was positively and highly significantly associated with CVG (0.91**), SVI (0.72**), GRI (0.80**) and SGI (0.95**). Also, MGT was positively and significantly correlated with RSGR (0.61**), but significantly and negatively associated with SGI (-0.35**) and GRI (-0.49**). It was also observed that CVG was positively and significantly correlated with SVI (0.70**), SGI (0.82**) and GRI (0.65**). A positive and significant correlation was also observed between SVI and SGI (0.60**), GRI (0.46) and RSGR (0.65**) as well as between GRI and SGI (0.95**).

Table 5: Pearson coefficient of correlation between pairs of seed germination indices and seedling growth characters across the experiments

	FGP	MGT	CVG	SVI	SGI	GRI	RSGR
FGP	-	-0.11	0.91**	0.72**	0.95**	0.80**	0.12
MGT		-	0.01	0.15	-0.35**	-0.49**	0.61**
CVG			-	0.70**	0.82**	0.65**	0.18
SVI				-	0.60**	0.46**	0.65**
SGI					-	0.95**	0.00
GRI						-	-0.04
RSGR							-

**, ** Significant at (P= 0.05) and (P= 0.01) respectively*

FGP: Final germination percentage; MGT: Mean germination time; CVG: Coefficient of velocity of germination; SVI: Seedling vigour index; GRI: Germination rate index; RSGR: Relative seedling growth rate; SGI: Speed of germination index

4. DISCUSSION

Seed germination depends on both external and internal factors around the seed [18]. The significant response of rice seed to varied concentration of humic substance and duration of priming suggests that seed germination indices and seedling growth characters of rice is dependent on many factors. The germination rate index (GRI) and seedling germination index (SGI) that decreased as the priming concentration increases in this study agrees with the finding of [19] who reported that with increasing concentrations of plant growth regulator, seedling growth and germination percentage of cowpea traits decreased dramatically, while other characters (SVI and RSGR) were inconsistent as the concentration increases. Comparatively, there was no significant difference between control (no treatment) and priming concentration regimes for FGP and CVG. This implies that the rapidity of germination of the seed lot was not improved by the priming treatment as none of the treatment performed significantly better than the control. This observation corroborates the work of previous scientists [20, 21] who reported that plant growth regulator (ComCat^{TM}) concentrations did not have significant effect on coleoptile growth of maize compared to the untreated control. On the other hand, it was observed that the seed lot without priming (control) had significantly low values as compared to the primed seed lot for MGT, SVI and RSGR. This suggests that seed treatment with humic substance can be used to improve the seedling vigour during seedling growth stage.

The GRI reflects the percentage of germination on each day of the germination period. Higher GRI values indicate higher and faster germination [22]. The GRI and SGI of rice seed lot that was not primed had better performance than primed seed lot. However, highest means recorded in MGT, SVI and RSGR of rice seed primed with 100% concentration agrees with the result of [23] who reported that priming of seeds with low concentration of Gibberellic Acid (GA_3) had no effect on seed germination while higher concentration of GA_3 increased shoot and root lengths, dry weight, fresh weight and tissue water content of maize.

It can be deduced that 4 hours of priming favoured most of the seed germination indices and seedling growth traits studied. This indicates priming rice seeds for 4 hours significantly improved most of the characters in this study. This shows that seed germination indices and seedling growth of rice can be improved by the duration of priming or seed soaking time, because four hours priming resulted in higher mean values for most of the characters in this study. [24] reported stimulatory effects of priming on the early stages of germination process with mediation of cell division in germinating seeds. Priming may improve germination by accelerating imbibition, which in turn would facilitate the emergence phase and the multiplication of radicle cells [25]. It can be deduced that 4 hours of priming favoured most of the seed germination indices and seedling growth traits in this study

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The significant difference observed in the reaction of the rice seed to humic substance priming in the two experiments shows that the first experiment had higher overall mean values that were significantly different from the second experiment for all of the characters measured in this study (FGP, CVG, SVI, GRI, RSGR and SGI) except MGT. However, the trend of the result in the result obtained in the two experiments were similar, hence the situation may be attributed to both internal and external environmental factors during the conduct of the experiments and may not be necessarily due to reaction of the seeds to the treatment.

Simple correlation analysis has been considered adequate as a rough guide to the magnitude and direction of the relationships between two traits [14]. Therefore, positive and significant correlation observed among the pairs of seed germination indices and seedling growth characters in this study suggests that these characters can aid in selection during rice improvement programs. [14] reported that significant positive correlation indicates that selection for one character could be used to indirectly select for another character. This study showed that seeds with high germination value will positively influence the other seedling growth parameters.

5. CONCLUSION

Rice seed priming with PGR (VimpelTM) did not significantly improve seed germination but the growth rate is influenced with the priming. Rice seed priming at 50% concentration for 4 hours significantly improve the seedling growth rate of rice. This study, therefore indicate priming rice seed with humic substance will improve seedling growth and vigour of rice under upland and lowland cultivation

COMPETING INTERESTS

No competing interests exist.

REFERENCES

1. J.K. Ladha, F.J. de Bruijn and K.A. Malik.: Introduction Assessing opportunities for nitrogen fixation in rice – a frontier project. *Plant and Soil*. 1997; 194:1–10.
2. Muthayya S, Sugimoto J. D., Montgomery S, Maberly G. F. An overview of global rice production, supply, trade, and consumption. *Annals of the New York Academy of Sciences*. 2014; 1324: 7–14.
3. Sahrawat KL. Iron toxicity in wetland rice and the role of other nutrients. *Journal of Plant Nutrition*. 2004; 27, 1471-1504.
4. Gbadegesin, RA, Adybeton, JO, Onyibe LE, Keki PK., Amos TT, Yusuf, JO, Omenaza ZE. Evaluation of the extent of adoption and impact of improved technologies on maize, rice and cassava production in the North West zone of Nigeria. *Nigerian Journal of Agriculture Extension*. 2012; 14 (12): 1-12.
5. Serpil S. Investigation of effect of chemical fertilizers on environment. *APCBEE Procedia*. 2012; 1: 287 – 292.
6. Basra MS, Dhillon R, Mali KC. Influence of seed pre-treatment with plant growth regulators on metabolic alteration of germinating maize embryos under stressing temperature regimes. *Annals of Botany*.1989; 64:37-41.
7. Kaya MD, Okcu G, Atak, M, Cikili Y, Kolsarici O. Seed treatments to overcome salt and drought stress during germination in sunflower (*Helinthus annuus* L.). Eur. *Journal Agronomy*. 2006; 24: 291- 295.
8. Basra SM, Farooq AM, Tabassum R, Ahmad N. Physiological and biochemical aspects of pre-sowing seed treatment in fine rice (*Oryza sativaL.) Seed Sci. Technol*. 2005; 33: 623- 628.
9. Inglis D, Du Toit L, Miles, C. : Organic seed treatments. Progress report: Organic cropping research for the Northwest. Washington: Washington State University & North Western Washington Research and Extension. 2004.
10. Trevisan S, Francioso O, Quaggiotti S, Nard S. Humic substances biological activity at the plant-soil interface. *Plant Signaling and Behavior.* 2010; 5 (6): 635–643.

11. Calvo P, Nelson L, Kloepper J.W. Agricultural uses of plant bio-stimulants. *Plant Soil* 2014; 383: 3 – 41.
12. Van der Watt E, Pretorius JC. In vitro and in vivo bio-stimulatory properties of a Lupinus albus L. seed suspension. *Crop Pasture Sci*. 2011; 62, 189–197. 10.1071/CP10391.
13. ISTA. International Rules for Seed Testing Association (ISTA), Zurich, Switzerland .2003; pp: 1-121.
14. Adebisi MA, Okelola FS, Alake CO, Ayo-Vaughan, MA, Ajala MO. Interrelationship between seed vigour traits and field performance in new rice for Africa (Nerica) genotypes (*Oryza sativa L.*). *Journal of Agricultural Science and Environment.* 2010; 10 (2):15-24.
15. Rajput A, Rajput, SS, Jha G. Physiological characters, leaf area index, crop growth rate, relative growth rate and net assimilation rate of different varieties of rice grown under different planting geometries and depths in System of Rice Intensification (SRI). *International Journal of Pure and Applied Bioscience*. 2017; 5 (1) 362-367.
16. AOSA. Rules for Testing Seeds, Association of Official Seed Analysts, Las Cruces, NM. 2003
17. IBM SPSS Statistics for Windows, (Version 23.0). Armonk, NY: IBM Corp. 2015
18. Ibrahim ND, Bhadmus Z, Singh A. Hydro-priming and re-drying effects on germination, emergence and growth of upland rice (*Oryza sativa* L.). *Nigerian Journal of basic and Applied Science* 2013; 21(2): 157-164.
19. Audi A, Muhktar F. Effect of pre-sowing hardening treatments using various plant growth substances on cowpea germination and seedling establishment. *Bayero Journal of Pure and Applied Sciences* 2009; 2:44-48.
20. Agraforum.Technical data sheet. Agraforum AG pty Ltd .german:Bomlitz. 2006.
21. Pholo M, Seef-Pretorius CJ. Seedling growth of Maize (*Zea mays L.)* response to seed treatments. *Biological Forum - An International* Journal. 2011; 3 (1):4-9.
22. Kader MA. A comparison of seed germination calculation formulae and the associated interpretation of resulting data. *Journal and Proceedings of the Royal Society of New South Wales.* 2005; 138: 65-75.
23. Ghodrat V and Rousta M.J.: Effect of priming with gibberlleric acid (GA3) on germination and Growth of Corn (*Zea mays L.*) under Saline Conditions. *International Journal of Agriculture and Crop Science.* 2012 ; 4(13) : 882-885.
24. Sivriteps N, Sivritepe, HO, Eris, A. The effects of NaCl priming on salt tolerance in melon seedling grown under saline condition. *Scientia Horticulturae*. 2003; 97: 229-237.
25. McDonald MB. Seed deterioration: physiology, repair and assessment. *Seed Science and Technology*. 1999: 27: 177-237.