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A Role of Weed Solvents on Seed Priming of Diverse Rice Germplasm Lines

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Rice (*Oryza sativa* L.) is the fastest growing staple food and cereal crop in Pakistan. Seed priming is a low-cost, low-risk technique that is easily adopted by resource-poor farmers in developing countries and increases the yield of crops. It improves seed performance, through increased seed vigor subsequent to enhanced percentage of seed germination and rates of seedling emergence. The effect of seed priming on germination percentage and mean time to germination of local and exotic cultivars of rice was studied. Effect of methanol leaf extract (10:100 w/v) of some weeds *Cyperus esculentus* (Della), *Axonopus compressus* (Itsit), *Convulvulus arvensis* (Lehli) and *Parthenium hysterophorus* (Parthenium) was assessed for their effect on rice seedling by laboratory experiments. In each Petri plate, ten seeds were placed and germination percentage and germination index of rice seeds was calculated. Weeds extracts priming increased germination rate and germination percentage as compare to control. An interaction affect on germination performance between vigor and priming was observed. The interaction between *C. esculentus* + *P. hysterophorus* showed increased germination percentage and growth enhancement in rice seedlings. Results showed that CDR448 (96%), Basmati-140 (84%), Begum-370 (80%) and Presidio (75%) were increased germination significantly among rice varieties. Conversely the seedling growth of Palman 60, Jhona-145, Ru8703196 and MAHIYA were least affected by weed extracts as compare to control. On the contrary, seed primed with weed extracts was non-significant in case of Raxmonth. The overall study indicated that seed priming technique with weed extracts proved to be the most effective to germination and seedling growth of rice.

Key words: Seed-priming, Rice, Seedling vigor, Germinatio, Weed extracts.

Seed become decent during storage condition because of unfavorable factors. Seed deterioration may have been occurred due to delay between collection and processing if seeds are held under inappropriate conditions, during processing or whilst in storage. Moreover, natural compounds contained by seed tissues will also face some damage in normal storage circumstances. However injury on seed will be repair if damage is not severe[1]. In previous literature seed viability and its vigor are the most important factor in the establishment of crop yield. In agriculture, a vigorous early seedling growth with well developed root systems can better withstand adverse conditions and associated with higher yields[2]. Seed priming is a technique in which seed vigor can be improved by enhancing the speed and uniformity of germination[3]. Furthermore osmo-priming is a special type of seed priming in which seeds are soaked in aerated low water potential[4]. It has been used to invigorate many horticultural[5] and agronomic crops[6]. In osmo-priming seeds allow pre-germinative activities to proceed, followed by re drying before actual germination that improves

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seed germination performance and stress tolerance[7]. Primed seed showed more uniformity, stability, distinctness, early seedling growth and ultimately enhance the yield potential of the crop in comparison with the unprimed seed[8].

Rice is the economically important crop of Pakistan and stand second position in cereal cultivation around the globe. It is essential constitute of staple food for 2.7 billion people worldwide and about 90% of the world's rice is grown in Asia[9]. Weeds and its parts including leaf, stem, root, and fruit can also effect a crop's growth by releasing allelochemicals into the growing environment[10,11,12]. Therefore extracts of different weeds also used in seed priming technique which shows different behavior in exerting their allelopathic effects on crops[13]. Seed germination and crop yield also increase with the application of weed extracts by releasing water-soluble compounds into soil[14]. But no research has yet been conducted on osmo-priming with weed extract. Therefore main objective of present findings was to evaluate the various physical parameters of local and exotic lines of rice varieties. In addition, to evaluate the impact of osmo-priming techniques with respect of weed (*C. esculentus, A. compressus, C. arvensis* and *P. hysterophorus*) extracts on the germination and seedling vigor of rice.

MATERIALS AND METHODS

Sample of 15 local varieties of rice viz., Hansraj 23A, Palman -60, Hansraj 197, LAL DHAN 304, MUSHKAN 340A, Basmati 140, ROHDU 150, CHIPET-A-200, CHAKLALA 201, CHAKLALA 201, DHAN KASARWALA 102-4, JHONA 145, BEGUM 135 and Basmati -370 were obtained from Rice Research Institute, Kala Shah Kaku, Lahore and stored in bags of 250 grams. Exotic rice germplasm of 15 different lines of rice viz., EKARIN, MAHIYA, IBASMATI-802, RONDO, NEWBONNET, 85HB0138C, CDR 448, CDR 210, REXMONTH, RU8703196, 85HB013BC, JASMINE 85, PRESIDIO, SABINA and GULFMONTH were obtained from United States Department of Agriculture, USA (Table 1).

Selection of Seeds

Seeds from each rice samples were randomly selected and tested for different physical traits (seed length, seed width, seed thickness, length/ width, and 1000 seed weight) and germination tests.

Measurement of Physical Characteristics of Local and Exotic Germplasm lines

Physical characteristic of 15 local varieties and 15 exotic varieties of rice were measured. For this purpose, 1000 seed weight for each sample was measured on a digital weighing balance, and three readings were taken for each sample. Seed length, seed width and seed thickness of these local rice varieties was measured with the help of a digital Vernier Calliper. For each sample of rice, these measurements were taken in three replicates; in each replicate 5 seeds were measured. The seeds were randomly selected from the seed samples.

Collection of Weed and Extract Preparation

The dominant weed species of *C. esculentus*, *A. compressus*, *C. arvensis* and *P. hysterophorus* were collected (aerial parts at maturity) from different fields of Lahore. Then leaves of each weed were washed, shade dried and finely ground with mortal and pestle. Powder of each shade dried weed materials (10 g) was put in the thimble and extracted successively with 100 mL methanol solvent for 48 h by using a soxhlet extractor. Then extract was concentrated using rotary flash evaporator, weighed and preserved in airtight bottles at 4ºC until further use[15]. The prepared extracts of *A. compressus* + *C. arvensis* (concentration-1) and *C. esculentus* + *P. hysterophorus* (concentration-2) are mixed in DMSO and made two concentrations.

Osmo-priming

Heathly, uniform and sterilized 30 seeds of Pressidio, Sicrra, Rexmont, Ru8803072, CDR448, CB-36, CB-39, Shaheen basmati, CB-21 and Sufaida were selected. Seeds were soaked in the weed extract for 30 minutes. The ratio of seed weight to solution volume was 1:5 (g mL^{-1})[16]. For control, seeds were soaked in distilled water. Afterward, soaked seeds were washed with distilled water and dried in laminar flow[17]. Then polythene bags were used for sealing the dried seeds and stored in refrigerator for further use.

In vitro, Germination and early seedling growth bioassays

Germination and early seedling growth of rice was studied with two concentrations of different weed extracts in lab experiment.

The layer of filter paper was lined in Petri dishes (9-cm dia.) then ten primed seeds were placed in each plate. Subsequently, seeds were moistened with distilled water daily. Three replicates of each treatment were arranged in a completely randomized design. Germination was recorded daily up to 10 days. After 3 days, the seedling length was determined.

Measurement of growth parameters

At the end of the experiment, germination rate was determined weekly by using the formula (Germinated seed / Total seed × 100) for each replication of the treatment[18]. The length of radicle and plumule were measured in centimeters from the point where the radicle and plumule joins together at the end of the radicle and to the top of the plumule, in addition fresh and dry weight were also measured by using the method of Tanveer *et al.* [(19)]. All measurements were done in three replicates and the mean calculated.

Statistical analysis

The data was analyzed statistically using Variance (ANOVA) followed by Duncan's Multiple Range Test[20] at pd"0.05 to compare treatment means.

RESULTS

In present study various physical parameters of local and exotic lines of rice varieties were studied. Moreover, seed priming technique exhibited momentous effect on seedling vigor and start of emergence of rice seeds. Different weeds (*C. esculentus*, *A. compressus*, *C. arvensis* and *P.*

Table 1. Plant Material

Sr.No	Name	Origin	Accession Number	Taxonomy
1	EKARIN	Myammar, Rangoon	GSOR311729	*Oryza sativa*
2	MAHIYA	FIJI	GSOR311753	*Oryza sativa*
3	IBASMATI-802	Pakistan	GSOR311216	*Oryza nivara*
4	RONDO	United State	PI657830	*Oryza officinalis*
5	NEWBONNET	United State	PI475833	*Oryza rufipogon*
6	85HB0138C	United State	PI500072	*Oryza sativa*
7	CDR 448	China	PI615009	*Oryza sativa*
8	CDR 210	China	PI615010	*Oryza sativa*
9	REXMONTH	United State Texas	PI502968	*Oryza sativa*
10	RU8703196	United State Texas	PI583278	*Oryza sativa*
11	85HB013BC	United State	PI527072	*Oryza sativa*
12	JASMINE 85	United States	PI657830	*Oryza sativa*
13	PRESIDIO	United States, Arkansas	PI590419	*Oryza sativa*
14	SABINA	Cunited State	PI636466	*Oryza sativa*
15	GULFMONTH	United States, Texas	PI502967	*Oryza sativa*
16	Hansraj 23A	Subcontinent		*Oryza sativa*
17	Palman -60	Subcontinent		*Oryza sativa*
18	Hansraj 197	Subcontinent		*Oryza sativa*
19	LAL DHAN 304	Subcontinent		*Oryza sativa*
20	MUSHKAN 340A	Subcontinent		*Oryza sativa*
21	Basmati 140	Subcontinent		*Oryza sativa*
22	ROHDU 150	Subcontinent		*Oryza sativa*
23	CHIPET-A-200	Subcontinent		*Oryza sativa*
24	CHAKLALA 201	Subcontinent		*Oryza sativa*
25	CHAKLALA 201	Subcontinent		*Oryza sativa*
26	CHAKLALA 211	Subcontinent		*Oryza sativa*
27	DHAN KASARWALA 102-4	Subcontinent		*Oryza sativa*
28	JHONA 145	Subcontinent		*Oryza sativa*
29	BEGUM 135	Subcontinent		*Oryza sativa*
30	Basmati -370	Subcontinent		*Oryza sativa*

hysterophorus) extracts were used for seed priming, these allelochemicals were extracted and released in a natural way (Figure 4). Results exhibited that the seed length, width, thickness, length/width and 1000 seed weight of the local and exotic varieties of rice were measured and it was clear that CDR448 (10.36mm) has the healthiest looking grain with husk among fine and coarse rice varieties used for

Table 2. Physical Parameters of different lines of Rice seeds (*O. sativa* L

S.V	DF	SL	SW	ST	L/W	1000GW
Genotypes	29	1.0084**	0.13897**	0.05959**	0.6950**	34.1827**
Replications	2	0.0512	0.00275	0.00717	0.0425	0.0498
Error	58	0.0215	0.00315	0.00440	0.0142	0.0718

Level of Significance: P<0.05=* P<0.01=**
*= significance, **= highly significance

Table 3. Simple Correlation among different seed physical parameters of rice (*O. sativa* L.)

Traits	SL	SW	ST	L/W	1000GW
SL	1.00				
SW	-0.078ns	1.00			
ST	0.095*	0.390**	1.00		
L/W	0.245**	-0.236**	0.132**	1.00	
1000GW	0.293**	0.490**	0.489**	0.041ns	1.00

Level of Significance: P<0.05=* P<0.01=**
*= significance, **= highly significance, ns= Non significant

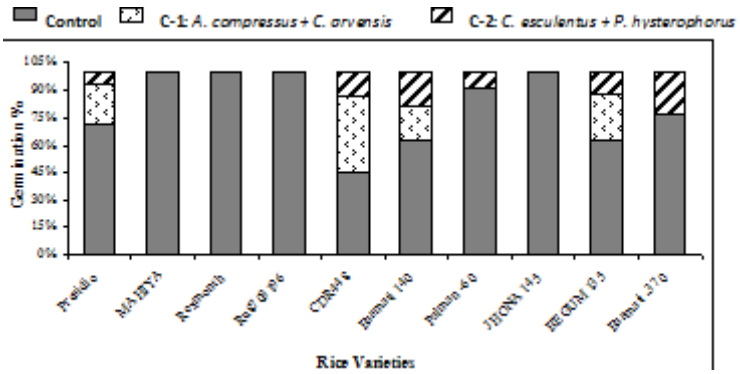

Fig. 2. Germination rate of different lines of Rice seeds after 6 days

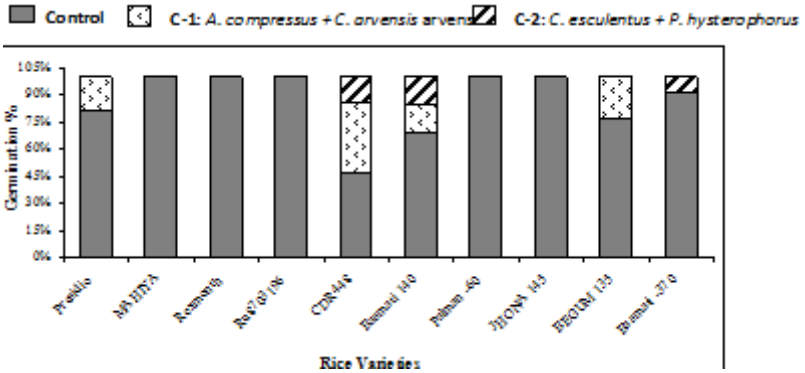

Fig. 1. Germination rate of different lines of Rice seeds after 3 days

Table 4. Two way anova of mean square values of various rice seedling traits at various concentrations

Sov	DF	Control				Concentration-1				Concentration-2			
		PL	RL	FW	DW	PL	RL	FW	DW	PL	RL	FW	DW
Genotypes	9	1.63**	3.28**	0.027**	1.14**	6.32**	7.05**	0.27**	0.0002**	5.19**	0.46**	0.32**	7.66**
Replications	2	0.112	0.338	0.0003	8.00	0.060	0.032	0.002	0.0012	0.364	0.004	0.112	7.20
Error	18	0.682	0.864	0.0004	8.70	0.219	0.308	0.002	0.0001	0.327	0.027	0.012	1.09
CV	14.99	22.12	5.86	7.42	17.26	34.36	17.99	31.24	34.34	26.80	13.82	21.07	
S.E	0.184	0.207	0.004	6.59	0.104	0.124	0.011	0.002	0.127	0.037	0.024	0.002	

Level of significance p<0.05=*, p<0.01=** and ns = non significant. PL; Plumule length, RL; Radical length, FW; Fresh weight, DW; Dry weight, Concentration1: *A. compressus* + *C. arvensis* and Concentration2: *C. esculentus* + *P. hysterophorus*

Table 5. Correlation studies of different rice genotypes seedling traits at various concentrations

Traits	Control				Traits	Concentration-1				Traits	Concentration-2			
	PL	RL	FW	DW		PL	RL	FW	DW		PL	RL	FW	DW
PL	1.00				PL	1.00				PL	1.00			
RL	-0.017**	1.00			RL	0.229**	1.00			RL	0.353**	1.00		
FW	-0.087ns	0.028ns	1.00		FW	0.053ns	0.067ns	1.00		FW	-0.006ns	0.001ns	1.00	
DW	0.101ns	-0.096ns	0.038ns	1.00	DW	0.044ns	-0.002ns	0.101ns	1.00	DW	0.030ns	0.051ns	-0.040ns	1.00

Level of significance p<0.05=*, p<0.01=** and ns = non significant. PL; Plumule length, RL; Radical length, FW; Fresh weight, DW; Dry weight, Concentration1: *A. compressus* + *C. arvensis* and Concentration2: *C. esculentus* + *P. hysterophorus*

study; since it has longest grain, equal in seed width and thickness with Begum-370 (2.93mm and 2.92mm). Among fine grain Basmati-140 had highest 1000 seed weight (30.2g) whereas Ru8703196 had the least weight of 17.2g (Table 2 & 3). Weed extract priming treatments significantly affected the germination percentage of rice varieties treatments *in vitro* as conditions (Figure 1-3). Furthermore, finding of this study were demonstrated that seed priming technique caused a significant increase among rice varieties: CDR448 (96%), Basmati-140 (84%), Begum-370 (80%) and Presidio (75%). On the other hand the seedling growth of Palman 60, Jhona-145, Ru8703196 and MAHIYA rice varieties were least effected by weed extracts as compare to control. The different solvent extracts of weed used in this study showed significant effects on germination and seedling vigor of rice seeds while

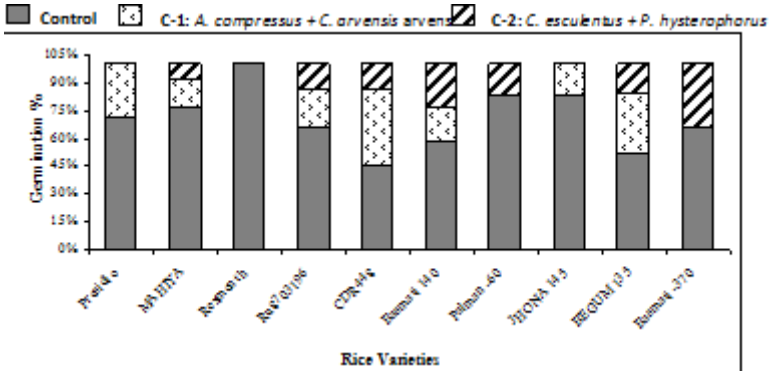

Fig. 3. Germination rate of different lines of Rice varieties after 10 days

Fig. 4. Allelopathic affect of various weed extracts solvent on early growth stage of various rice seeds and physical parameters measurement of rice germplasm lines (local + exotic)

in some treatments exerted non-significant results (Table 4 & 5). In the case of CDR448 (96%), C-1 of weeds revealed better rate of germination as compare to other treatment combination. Conversely the lowest germination rate was observed in MAHIYA variety while Ru8703196 and Presidio showed moderate rate of seed germination. Pre-sowing soaking by weed extracts proved to be the most effective to germination and seedling growth of rice. The overall results indicated the possible supportive effect of allelochemicals present in tested weeds on rice seeds. Conversely, weed extract osmoprimed seeds with C-1 showed maximum germination in case of Basmati-370 and Palman-60. It was also observed that in both weeds treatments (C-1 and C-2), extract treated seedling did not survive in Rexmonth variety. These findings also supported that different concentration of natural bio-molecules present in weed extracts. Further studies on these bio-molecules are important for checking the weed effect during seedling stage in field.

DISCUSSION

The length, width, seed thickness, length width ratio and 1000 grain weight is one of the quantitative measures for grain size and grain shape. Grain morphology i.e. colour, size and shape having unique position for the breeders during the selection and evaluation process[21, 22]. In the present study all the genotypes showed significant variations with respect to their desirable seed morphological traits. It is thought to relate the largest shape variation in small grain crops. On the other hand, length width ratio is the major genetic variation of rice grain shape and highly associated with the quantitative traits parameters and can be used in the breeding program for the improvement of the rice varieties[23, 24]. The results exhibited that seed length, width, thickness, length/width and seed weight is highly significance because the values are less than 0.01 and 0.05 by using variance. It may also involve in seed health and vigour that leads to increase the germination rate and ultimately increase the yield potential and also important for the allelo chemicals studies that produce from the various rice varieties. It is also a sign of healthy crop and good seeing to the scientist and farmers community. Furthermore results demonstrated that the seed width and seed length have negative correlation and non-significant. Positive and negative correlations both important for plant biology study. Although it was a preliminary laboratory study yet it provided encouraging results and basis for future research. This may be important in increasing the germination and seedling growth of rice varieties in field and establishment of sustainable agriculture.

In vitro experiments, different weed extracts produced significant effect and improved emergence percentage by seed priming technique. The priming-improved seed performance might be attributable in part to the decreased lipid peroxidation and increased antioxidative activities during seed imbibitions. These results are in accordance with the results of other researchers who reported either improvement of germination percentage[25]. Also Lin and Sung [(26)] reported that priming the bitter gourd seeds before sowing overcame sub-optimal environmental effects on germination subsequent seedling establishment performance. Wang *et al.,* [(27)] reported that, both hot water-soaked and primed seeds showed significant increase in germination performance. The results are in line with the findings of Thornton and Powell [(28)] in Brassica and Srinivasan et al., [(29)] in mustard. These results agree with Amador et al., [(30)] in cowpea, they affirmed that drought or salinity may influence germination by decreasing the water uptake and toxicity of ions. The role of weed solvents in seed priming is a more effective tool to increase the growth, vigor and germinability of the seed. On the other hand, this technique may be helpful for the improvement of root growth, shoot growth and yield of the rice crop.

These findings matched with studies on watermelon in which emergence force and seedling growth were strengthened by seed priming[31]. Osmo-priming with weed extract had significant effect on the germination, seedling vigor and in rice varieties used in the present investigation (Table 4-5). Response of rice seeds to different osmo-priming treatments was significant and higher radicle and plumule length root and shoot length as observed in treated seeds might be the result of earlier germination and emergence (Figure 1-3). Osmo-priming has been proved to improve germination rate and speed in rice seeds especially when freshly harvested seeds are used[32]. Other

workers supported that osmotically primed seeds showed improved stand establishment, early seedling growth, seeds emerged earlier and more uniformly than seedlings from untreated seeds[33]. The functional characteristics of priming are primarily due to pre enlargement of the embryo[17], and improvement of germination rate[34]. Generally, in studies with aqueous extracts, the observed inhibitory effect are attributed to change in pH and osmotic potential thereby raising concern about allelopathy and its ecological existence and relevance[10]. On the other hand treatments of C-1 solvent extracts significantly showed germination as compare to other but these findings was contrast with Kazinczi et *al*.[(35)] that investigated *C. arvensis*, effect germination and seedling growth in of tomato and wheat and fresh weight of wheat, maize, oilseed rape, sunflower and tomato, but the fresh weight of sugar beet was stimulated. However, C-1 priming treatments resulted in improved seedling shoot length than of control, whereas C-2 treatments were effective in root growth. On the same line, higher growth stimulatory effect of *Cassia tara* and *Chromolaena odorata* aqueous extract was recorded on rice verities[36]. Swaminathan *et al*. [(37)] in their studies also observed the positive effect of leaf extracts on rice radical growth. But, a significant increase in shoot length of rice seedlings previously treated with *C. esculentus* and *A. compressus* is in contradiction with the results of Oudhia [(38)] and Veenapani [(39)], who observed significant reduction seed germination and seedling growth in Chickpea. The results of rice seedling root and shoot length indicated that, weeds extracts have varying degree of inhibitory and stimulatory effect. From the present investigation it may be concluded that germination and seedling vigor can be enhanced by osmopriming treatments with weed extracts in rice cultivars. However weed extract priming is new but can be an effective idea. The present study throws some light for further detailed studies on this aspect.

CONCLUSION

The information generated from study is to enhance the germination, seed vigor, plumule and radicle growth of various varieties of rice seeds by using different weed solvent extracts i.e. *C. esculentus*, *A. compressus*, *C. arvensis* and *P. hysterophorus*. Primed seed may be also helpful for the improvement of uniformity, stability, distinctness, early seedling growth and ultimately enhance the yield potential and disease resistance of the rice crop. Therefore, such type of pre-sowing treatments may be used to test seed viability for long storage in future breeding experiments. The results could be used for the production of rice varieties on large scales which is equally beneficial to the plant biologist scientists and farmers community by applying solvent extracts of different weeds. Further investigations are also needed to identify the various active compounds and their composition that are involved in seed priming.

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