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Enhancing the Vigour and Viability of Aged Seeds through Extract of Grapes (*Muscat hamburg)*

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A B S T R A C T

Indian farmers often save seeds for sowing in the next season, particularly the seeds of paddy, and pulses. However, these reserved seeds tend to lose their vigour and viability over time, resulting in a decrease in yield. To address this issue, a simple seed treatment using antioxidant substances can be effective. In the present study, grape juice and grape seed extracts, which are rich in antioxidants, were used at different concentrations to treat aged seeds of paddy and green gram. The treated seeds were germinated using the roll towel method to assess various germination traits. Extracts of grapes seed were extracted through acetone extract method recorded the highest seed quality and biochemical parameters. Laboratory experiments revealed that seed treatment using grapes seed extract at 2.5 % concentration was found effective for maintenance of seed vigour and viability parameters. The advantageous effect was evident through seed quality attributes *viz.,* speed of germination, germination percent, root length, shoot length, dry matter production and vigour index. It was also mirrored in pot culture and biochemical parameters pertaining to seed vigour and viability.

Introduction

India is abundant in cereals and pulses, which are vital sources of carbohydrates and proteins. These crops are among the most important in Indian agriculture. Rice (*Oryza sativa L.*) serves as the primary food for nearly half of the world's population. Pulses are particularly significant as they are the most important legume crops in India, known for their high-quality protein content. Green gram (*Vigna radiata L.*) is one of the most essential pulses in the country, providing an excellent source of high-quality protein with a digestibility of

around 25%. Leading Producer of Pulses In India is Madhya Pradesh (6.03) With Area of 5.59 Million hectares.

All of these physiological changes during seed deterioration have been attributed to various biophysical and biochemical changes in seed components, such as the loss of enzymatic activities, the loss of membrane integrity, accumulation of toxic substances and genetic alterations (Preistley, 1986; Hendry, 1993). However, the exact cause of loss of seed viability is still not well defined.

Lipid peroxidation and associated free radical oxidative stresses are considered to be major contributors to seed deterioration (Wilson and McDonald, 1986). However, different mechanisms of seed deterioration may exist under different storage conditions.

The primary phenolic compounds found in grape seeds are antioxidants, including flavan-3-ols, anthocyanins, flavanols, stilbenes, and phenolic acids. Antioxidants eliminate oxidative damage to a specific target molecule. This includes compounds that are both non-enzymatic and enzymatic in nature. Such organic molecules can aid in the preservation of seed viability as they age.

In light of the challenges faced by farmers, this study was conducted to explore a simpler solution using grape extracts. Specifically, the research involved treating aged seeds of rice and green gram with extracts from black grape juice and seeds. The objective was to improve the vigour and viability of these aged seeds. The findings indicate that the antioxidant properties of grape juice and seed extracts can enhance the vigour and viability of aged seeds, potentially addressing some of the issues farmers encounter.

Materials and Methods

The Paddy, and Green gram seeds were procured from the, Department of Agronomy, Agricultural College and Research institute, Coimbatore.

Grapes extracts preparation

The grapes berries (*Vitis vinifera L.*) were collected from Narmatha farm, Theni district, Tamil Nadu. The fruits get separated and were washed with fresh water. The obtained seed and skin were then shade dried for 4 – 5 days followed by sun drying for 2 – 3 days.

Extraction method

The grape seed and skin flour samples were extracted with two different solvents: 50% acetone and 0.5% (v/v) acetic acid. Samples were extracted in a ratio of 1 g to 10 ml (Parry *et al.,* 2006). The samples were centrifuged at 5000 rpm for 30 minutes. The extract was collected and stored. The filtrate constituted to 100 % stock solution. From the constituted solution, different concentrations of 2.5, 5.0, 7.5 were prepared. The Paddy and Green gram were primed with grape juice, skin and seed extracted at different concentrations, durations and soaking volume as follows.

After the soaking period, the excess solution was drained off, surface dried under shade for 24hr and dried back to original moisture content. The primed seeds were assessed for germination, seedling growth and biochemical parameters.

The laboratory experiment was conducted to enhance the vigour and viability of aged paddy seeds through seed treatment with grapes extract as per the standard procedure.

Treatment details

T_0 - Control
T_1 - Water soaking
T_2 - Grape seed extract - 2.5% (w/v)
T_3 - Grape seed extract - 5% (w/v)
T_4 - Grape seed extract – 7.5% (w/v)
T_5 - Grape seed extract – 10% (w/v)

Seed quality parameters

Speed of germination

Four replicates of one hundred seeds each were used to test the speed of germination of seeds. The seeds showing radicle protrusion were counted daily from third day of sowing until fourteenth day. From the number of seeds germinated on each day, the speed of germination was calculated using the following formula and the result was expressed in number (Maguire, 1962).

$$\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots\ldots\ldots + \frac{X_n - X_{n-1}}{Y_n}$$

X_1- Number of seeds germinated at first count

X_2- Number of seeds germinated at second count
X_n- Number of seeds germinated on n^{th} day
Y_1- Number of days from sowing to first count
Y_2- Number of days from sowing to second count
Y_n- Number of days from sowing to n^{th} count

Germination (%)

Germination test in quadruplicate using 400 seeds each with 4 replicates of 100 seeds were carried out in paper medium. The germination set up has been placed in a germination room maintained 25 ± 2°C and 95 ± 3 per cent relative humidity. At the end of the 14 days germination period recommended as per ISTA (2003), the seedlings were evaluated. Based on the mean number of normal seedlings developed, the mean germination was expressed in percent.

Root length (cm) and Shoot length (cm)

At the time of germination count, ten normal seedlings were selected at random from each treatment and replication, and used for measuring the root length of seedlings. Root length was measured from the point of attachment of seed to the tip of primary root using measuring scale.

The mean values were expressed in cm. The seedlings used for measuring root length were also used for measuring shoot length.

The shoot length was measured from the point of attachment of seedling to tip of the primary leaf and the mean values were expressed in cm.

Seedling dry matter production (g seedlings^{-10}) and Vigour index I

The normal seedlings used for growth measurement were placed in paper cover and dried under shade for 24 hr and then in a hot air oven maintained at 85°C for 24 h and the weight was recorded using an electronic balance. The mean weight was expressed as g seedlings^{-10}.

Seedling vigour index was calculated by adopting the following formula as suggested by Abdul-Baki and Anderson (1973) and was expressed in whole number.

Vigour index = Germination percentage x Seedling length (cm)

Biochemical parameters

Dehydrogenase activity

The dehydrogenase activity of the seeds was estimated following the method of Kittock and Law (1968). 20 seeds from each treatment were soaked in water for 12 hr. From this, five embryos were separated and incubated in darkness with ten ml of 0.2 percent tetrazolium chloride solution for two hour after incubation the tetrazolium chloride solution was decanted and the seeds thoroughly washed with distilled water and surface dried with blotters. The formazon was eluted by soaking the stained seeds in five ml of methyl cellosolve (2 methoxy ethanol) for two hour and the optical density was measured using Cary UV spectrophotometer at 470nm.

α amylase activity

Two grams of agar shreds and one gram of potato starch were mixed together in water to form a paste and the volume made up to 100 ml. The homogenous solution of agar starch mixture after boiling was poured into sterilized petridishes and allowed to settle in the form of gel after cooling. The pre-soaked paddy seeds from each treatment were cut cross sectionally (with their half endosperm and embryo portion intact) was placed in the petri dishes in such a way that the endospermic part remained in contact with agar-starch gel. The dishes were closed and kept in dark at 30°C. After 24 hours, dishes were uniformly poured with potassium iodide solution (0.44g iodine crystal + 20.008 g potassium iodide in 500 ml of 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 α amylase activity (Simpson and Naylor, 1962).

Experiment II

The laboratory experiment was conducted to enhance the vigour and viability of aged green gram seeds through seed treatment with grapes extract as per the standard procedure.

Treatment details

T_0 - Control
T_1 - Water soaking
T_2 - Grape seed extract - 2.5% (w/v)

T_3 - Grape seed extract - 5% (w/v)
T_4 - Grape seed extract – 7.5% (w/v)
T_5 - Grape seed extract – 10% (w/v)

Seed quality parameters

Speed of germination: as detailed in Experiment 1
Germination: as detailed in Experiment 1
Root length: as detailed in Experiment I
Shoot length: as detailed in Experiment I
Seedling dry matter production: as detailed in Experiment I
Vigour index I: as detailed in Experiment I
Field emergence: as detailed in Experiment I

Biochemical parameters

Dehydrogenase activity: as detailed in **Experiment I**
α amylase activity: as detailed in **Experiment I**

Statistical tools

The variability of different treatments was analyzed using a Completely Randomized Design (CRD).

Results and Discussion

Grapes seed and skin extraction with acetone: water was more effective compared to acetic acid: water extraction. As for solvents extracts, extraction with acetone: water (50:50) led to the maximum phenolic content and antioxidant capacity, while water gave the lowest phenolic content and antioxidant capacity. This result indicated that aqueous solution of acetone was better than a single compound solvent system for extraction of total phenolic from plant materials. Water, aqueous mixtures of ethanol, methanol and acetone are commonly used to extract plant materials. The extracting solvents significantly affect extraction yield, phenolic content and biological activities of plant materials (Jayaprakasha *et al.,* 2001; Jayaprakasha *et al.,* 2003; Spigno and Faveri, 2007; Lafka *et al.,* 2007).

The present investigation revealed that positive performance of grapes seed and skin extracts on physiological and biochemical attributes. Grapes seed extract at 2.5% concentration increased the speed of germination and germination percent in paddy and, green gram. This might be due to the anthocyanins and procyanidins which enhance the speed of germination and germination percent. This is in endorsement with the findings of Mattivi *et al.,* (2002) who reported red grapes contain procyanidins and anthocyanins occur both in the seeds and the berry skin had a positive effect on speed of germination and germination percentage due to the enhanced antioxidant efficiency by the seed priming. Seed priming, which enhance the seed vigour, speed and uniformity of germination (Demir and Van DeVenter, 1999). Similar such findings were reported in rice and wheat (Basra *et al.,* 2002, 2004), cotton (Afghani and Asl Taheri, 2012).

The longest root and shoot length were observed in the seeds treated with grapes seed and skin extract at 2.5% concentration compared to the control and water soaking seeds. This might be due to the antioxidant present in the grapes skin extract. A similar trend was reported by Ramalal *et al.,* (1993) seed priming significantly increased the germination, shoot length, root length, seedling vigour index and seedling dry weight of maize.

Table.1 The Paddy, and Green gram seeds were procured from the, Department of Agronomy, Agricultural College and Research institute, Coimbatore.

Sl. No	Crop	Variety	Age of the seed
1	Paddy	MDU 6	9 months old
2	Green gram	Co 6	3 months old

Extraction method

Crop	Treatment	Concentration (%)	Duration (hr)	Volume (ml)
Paddy	Grape seed	2.5, 5, 7.5, 10	12	1:2
Green gram			3	1: 0.3

Table.1a Effect of seed priming with grapes juice extracts on speed of germination, germination (%), root length (cm), shoot length (cm), Dry matter production and Vigour index in certain crop seeds

Treatments (T)	Speed of germination		Germination (%)		Root length (cm)		Shoot length (cm)		Dry matter production		Vigour index	
	Paddy	Green gram	Paddy	Green gram	Paddy	Green gram	Paddy	Green gram	Paddy	Green gram	Paddy	Green gram
T_0	8.27	10.46	74	75	24.40	16.40	12.80	26.50	0.0802	0.0900	2753	3218
T_1	9.38	11.26	72	83	23.30	16.70	12.30	27.70	0.0901	0.1200	2563	3685
T_2	13.57	12.27	85	85	27.00	17.70	14.70	28.60	0.1300	0.1400	3545	3936
T_3	10.48	10.86	74	82	26.60	17.00	13.30	27.70	0.1200	0.0800	2953	3665
T_4	11.37	10.26	62	74	25.30	17.00	13.40	28.10	0.1100	0.0900	2399	3337
T_5	12.18	11.36	72	70	24.80	16.50	13.70	27.80	0.1100	0.1200	2772	3101
Mean	**10.88**	**11.08**	**73.17**	**78.17**	**25.23**	**16.88**	**13.37**	**27.73**	**0.1100**	**0.1100**	**2831**	**3490**
SEd	0.249	0.216	1.418	2.258	0.585	2.876	0.182	0.637	0.003	0.004	52.776	70.168
CD(P=0.05)	0.548	0.475	3.124	4.975	1.289	0.396	0.401	2.814	0.006	0.004	116.262	154577

Table.2 Biochemical changes in different concentrations of grapes juice extracts on Dehydrogenase activity (OD value) and -amylase activity (mm) in certain crop seeds

Treatments (T)	Dehydrogenase activity (OD value)		α -amylase activity (mm)	
	Paddy	Green gram	Paddy	Green gram
T_0	0.1267	1.0672	7.70	11.45
T_1	0.1934	1.8340	8.10	11.83
T_2	0.2183	2.1360	9.40	12.73
T_3	0.2619	2.8431	10.76	13.82
T_4	0.2038	2.3680	9.70	12.03
T_5	0.2386	2.5194	8.60	11.47
Mean	**0.2071**	**2.1280**	**9.0433**	**12.221**
SEd	0.254	0.278	0.246	0.178
CD(P=0.05)	0.541	0.004	0.142	0.164

Treatment details

T_0	Control	T_2	Grape Seed – 2.5% (w/v)	T_4	Grape Seed – 7.5% (W/V)
T_1	Water soaking	T_3	Grape Seed – 5% (W/V)	T_5	Grape Seed – 10% (W/V)

Priming of sunflower normal or low vigour seeds improved the seedlings vigour in terms of radicle, plumule length and their root shoot ratio and fresh weight (Kausar *et al.,* 2009). The higher enzyme activities were observed in the seeds primed with grape seed and extract of 2.5% concentration in the crops of paddy, and green gram. Seed priming solutions containing inorganic solute, hormones or antioxidant compounds is helpful to express antioxidant defense genes which defend the cell against oxidative damage and lipid peroxidation (Saha *et al.,* 1990). This is in endorsement with the findings of Zhang and Heckly, 1993, Pal and Basu, 1994 they concluded that the peroxidase enzyme activity was to ascertain the

In conclusion, the extraction methods, the seed quality and biochemical parameters were maximum at acetone extract method and the minimum was recorded in acetic acid method. With respect to grapes seed extract, 2.5% concentration recorded maximum speed of emergence which reflected on highest germination percent than control seeds. The seeds primed with grapes seed extract at 2.5% concentration improved the resultant seed quality with better root length, shoot length, dry matter production and vigour index compared to unprimed seeds.

Author Contributions

R. Senthil Raj: Investigation, formal analysis, writing—original draft. K. Keerthi: Validation, methodology, writing—reviewing. M. A. Mohammed Vasi Ullah:—Formal analysis, writing—review and editing. K. Mohan Raj: Investigation, writing—reviewing. V. P. Nikitha: Resources, investigation writing—reviewing. S. Nivashini: Validation, formal analysis, writing—reviewing. V. Prabha Sree: Conceptualization, methodology, data curation, supervision, writing—reviewing the final version of the manuscript. S. Preethi: Validation, methodology, writing—reviewing. K. Saranya:—Formal analysis, writing—review and editing. S. Varshini: Investigation, writing—reviewing. E. Vignesh: Resources, investigation writing—reviewing. P. Vignesh: Validation, formal analysis, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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