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Original Article

$NaBH_4$ and APS as Novel Inducers of Seed Germination in Green Gram (*Vigna radiata*) and Black Gram (*Vigna mungo*)

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Abstract

igna radiata (mogumah) and *Vigna mungo* (matimah) seeds loose vigour due to high temperature, if the moisture content of the seed is high as it is in state of Assam. Therefore, vigor loss due to heat and moisture is a common phenomenon in the region. Germination and vigour index of seed treated with $NaBH_4$ and APS were found to be higher (38-60%) than that of the seeds without chemical treatment. APS (1mM) was optimum for the germination of black gram as well as green gram. In case of $NaBH_4$, 8mM was optimum for the germination of black gram and 1mM was optimum for the germination of green gram. We believe that the above two chemicals might be useful for stimulating germination of other seeds as well.

Keywords: Heat Stress; Vigour Index; Germination; Sodium Borohydride; Ammonium Persulfate; Synchronized Germination.

Introduction

Researchers reports about seed germination stimulators like karrikins, cyanohydrins etc.[1]. Mehanna et al. (1985) reported the role of hormones and chemicals on seed germinations [2]. One of the common methods of colloidal silver nanoparticle synthesis is using $NaBH_4$ as reducing agent [3]. The excess of $NaBH_4$ in the colloidal solution acts as stabilizing agent [3]. Recently we have observed that colloidal silver nanoparticle solutions stimulate germination and vigor of black gram seeds. Silver nanoparticle has shown some beneficiary effects on plant as reviewed by Nair et al. [4]. Therefore we thought that the above stimulatory effect on seed germination might be due to either silver and/or $NaBH_4$. There are reports of both stimulatory and inhibitory effect of colloidal silver nanoparticle on seed germination [5,6,7]. It has been reviewed that silver nanoparticles with stabilizer might be less toxic compared to that of silver nanoparticles without stabilizer [4,5,6,7]. Colloidal silver nanoparticles often have problems related to stability [8]. Dissociated silver from silver nanoparticles might also form the precursor $AgNO_3$ [3]. Silver nitrate enhanced the abscisic acid sensitivity in embryo and thereby might inhibit germination [9]. Considering all the above different studies relating to the role of silver on seed germination, we got curious to study the effect of $NaBH_4$ on seed germination with an aim to eliminate any possible role of $NaBH_4$ in it. Accordingly when we did the experiment to evaluate the role of $NaBH_4$, we found its stimulatory impact on green gram and black gram seed germination. Based on these interpretations a hypothesis is prepared that $NaBH_4$ might lead to increase in seed vigor. $NaBH_4$ is a strong reducing agent. Therefore, we tested the impact of a strong oxidizing agent like ammonium persulphate (APS) on seed germination. Interestingly, APS also found to be a germination stimulator for heat stressed seeds.

Vigna radiata and *Vigna mungo* are most common staple food in India and other countries of the world. In 2003-04 pulses produced were 635 kg/ha in an area of 23.46 million hectare that slightly decreased to 597 kg/ha grown in an area of 24.54 million hectare

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in 2008-09 as per report of Agriculture Ministry, Govt. of India [10]. As reported by Dubey for a high yield of pulses, instead of considerable improvement has been made in developing techniques, their production per hectare has remained the same for the last two centuries [10]. In India, 12 major different pulse crops are grown and *V. radiata* (green gram) and *V. mungo* (black gram) are among them. Therefore, studying seed germination of these two crops *V. radiata* (green gram) and *V. mungo* is going to be of significant importance. Murthy *et al.* reported the aging of *V. radiata* seed in terms of decrease in the vigour index [11]. They suggested that moisture content of the seed is directly proportional to vigour index loss due to heat stress. In their experiment, they have reported complete loss of vigour index due to loss of germination percentage within 30 days of treatment at 33^{O}C when water content was 0.222 g/g dry seeds weight. This suggests that even moderate temperature can deteriorate the seeds if stored under high humid conditions. Unfortunately, the relative humidity of North Eastern India is very high (60-80%) throughout the year [12]. Further, a vast majority of the population is rural (>80%) and below poverty line (36%) [12].The climate change is increasing the average temperature and North East India along with rest of the Indian subcontinent is experiencing 3-5^{O}C warming [13]. Another problem with heat stress is loss of synchronized germination. This becomes major problem with respect to fertilizer application, pesticide application and harvesting due to non-uniform developmental stages of plants. These environmental and socioeconomic conditions ensures that a huge part of farmers seeds lose their vigour index during storage. There are many chemicals used for stimulating germination of seed. Gibberellic acid stimulate embryo and promote germination [9]. Literature on use of germination stimulator for countering heat stress based loss of vigour index in mung and black gram are scanty [14]. Further, gibberelic acid being a plant hormone is not user friendly with respect to farmers use, storage, cost etc.

To address the above said problem and to find a new germination stimulator to counter heat stress based loss of vigour index in mung and black gram, a research is designed with an objective to evaluate the potency of sodium borohydride and ammonium persulfate.

Materials and methods

Materials and Chemicals

V. radiata (VR) and *V. mungo* (VM) seeds were obtained from local market. Sodium borohydride ($NaBH_4$) and ammonium per sulfate ($(NH_4)_2S_2O_8$) were of Merck, India. 2, 3, 5 Triphenyl Tetrazolium Chloride of AR grade were procured from Merck, India.

Imbibitions

Both seeds (*V. radiata and V. mungo*) of 25 seeds were washed and surface sterilized. These lots were subjected to soaking in sterile distilled water (25 ml) for different hrs from 0-24h with interval of 1h. The weight (g) of water absorbed per g of dry seed weight were measured in an hour wise manner from 1 to 24 hr. Average value of triplicate is presented graphically.

Heat Stressed Seed Production

Heat stressed seed production was performed using the method described by Murthy *et al.* [9]. Briefly, seeds of both species were allowed to imbibe water of around 0.222 g/g dry seed weight and incubated at 37^{O}C incubator (Orbitek-LE) for 144h for decreasing vigor index. After 72 hrs, the seeds having about 90% (*V. mungo*) and 60% (*V. radiata*) vigor index as compared to control, as presented in Figure S1, are used for chemical treatment assay using APS and $NaBH_4$ to counter vigour loss due to heat stress.

Stimulating Germination in Heat Stressed Seeds

Tweenty five seeds of both species were soaked in 25 ml volume of different concentrations of APS and $NaBH_4$ for 24hr. The concentrations of APS and $NaBH_4$ used were 1, 2, 4, 6, and 8 and 10mM.

- *Vigor Index Calculation*

The vigor index of the germinating seeds was calculated after 7 days of incubation at 25^{O}C under dark condition using the formulae:

Vigor index= Average plantlet length (cm) x germination (%)

- *Viability Assay*

Viability of seeds receiving optimum concentration of chemicals to show maximum vigor (for *V. radiata* 1mM APS and $NaBH_4$; *V. mungo*, 1mM APS and 8mM $NaBH_4$) were examined using the method described by Grzybowski *et al.* [15]. Briefly, treated seeds were soaked in 250 ml volume of 0.01% (w/v) 2, 3, 5 Triphenyl Tetrazolium Chloride for 24 h in dark, then washed with sterile distilled water and put in 100 ml of 95% (v/v) ethanol for distaining. Optical density of red color of formazan was measured at 480 nm against blank.

Biochemical Assay

Different biochemical assays were performed for total protein, reducing sugar, soluble starch and total polyphenols present in the seeds of optimum chemical treated concentrations. The seeds were imbibed in different treatments for 24 h along with controls. Protein estimation is carried out using Lawry's method [16]. Reducing sugar estimation is carried out using the procedure described by DNS method [17]. Starch estimation was carried out following I2/KI method [18]. Polyphenol estimation is carried out following Folin Ciocalteu method [19].

Results and Discussions

Water Imbibition and Heat Stressed Seed Production

Water imbibition is performed to obtain seeds containing 0.222 g/ g of dry seed weight. *V. radiata* (Supplementary Figure 1) has a more water imbibition capacity than *V. mungo*. *V. radiata* seeds needed only 1h to absorb 0.222g water/g of dry seed weight. On the other hand, *V. mungo* seeds needed 19h to imbibe the same amount of water. Water imbibition is known to be dependent upon the nature as well as the thickness of the seed coat [20,21]. These seeds containing 0.222g water/g of dry seed weight were subjected to heat treatment at 37°C for production of heat stressed seeds. The seed vigour losses due to heat treatment at 37°C for 144 h were presented in Supplementary Figure S2. It was observed that *V. mungo* vigour loss is quite slow compared to *V. radiata*.

V. radiata seeds vigour index decrease with increasing time period of heat treatment was smooth unlike that of *V. mungo* (Supplementary Figure 2). All *V. mungo* seeds did not imbibe uniformly. That might be reason behind the increase in vigour index on 24 and 96 h (Supplementary Figure 2). Collection of seeds

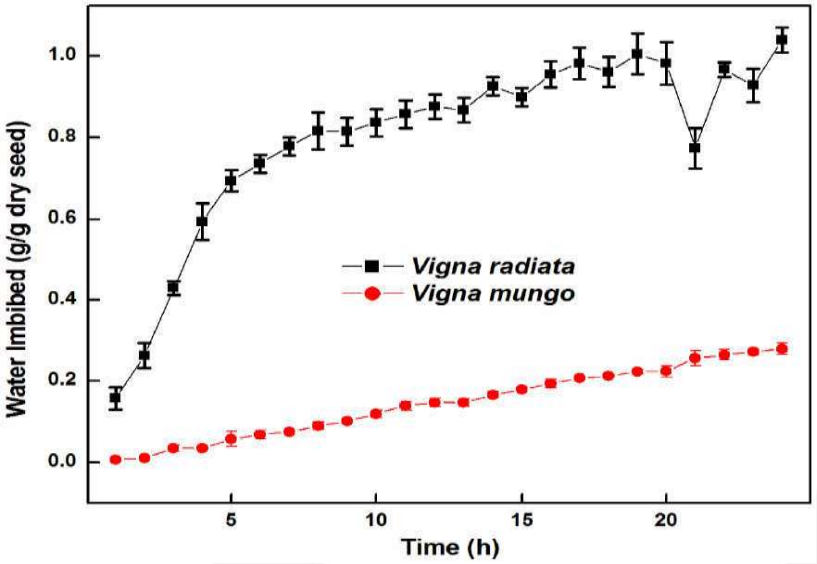

Fig. 1: Amount of water imbibed with reference to soaking for different time intervals

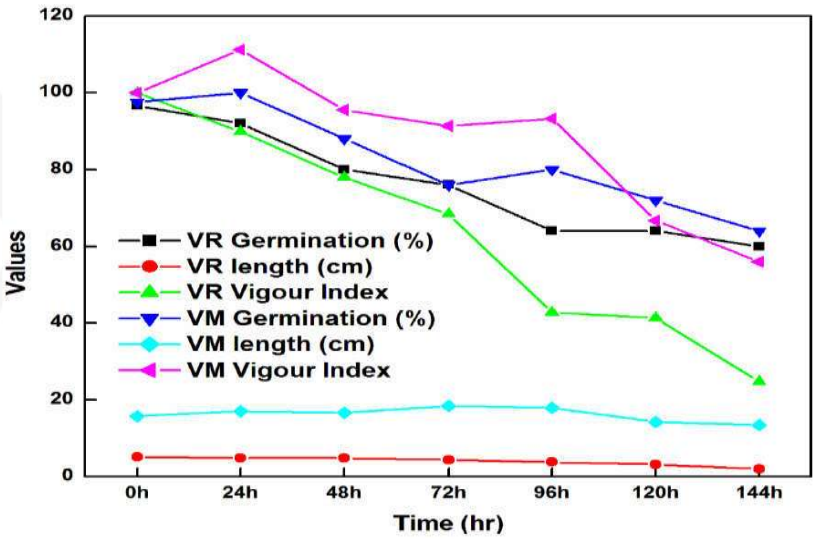

Fig. 2: Germination (%), vigor index and average plant length decrease due to heat treatment of seeds (0.222 g water/g dry seed) at 37°C for different time intervals. VM: *V. mungo*; VR: *V. radiata*.

for chemical treatment was performed after 72h heat treatment, when vigour index *V. mungo* was 90% and that of *V. radiata* was 60% (section 2.3.) (Supplementary Figure 2). The above seeds were stored at 20°C for chemical treatment using APS and $NaBH_4$. During chemical treatment experiment performed later, vigour index for the same seeds of *V. mungo* was recorded around 70-80% (Figure 3b, cyan and pink colour) and that of *V. radiata* was around 40-60% (Figure 3b, yellow and brown colour). The decline in vigour index might happen during storage at 20°C after heat stress. Therefore, it might be suggested that, once heat stress was presented to the seeds with the experimental moisture condition, even storage in non heat stress condition (20°C) cannot stop the loss in vigour. The difference can be realized by comparing the vigour index of seeds without any heat treatment (controls) in Figure 3.a with that of heat stressed seeds (Figure 3b).

Countering Heat Stress and Germination Stimulation

The germination and vigour index calculations are related to each other. Vigour index is a product of germination (%) and average plantlet length (cm). Vigour index is a complete representation of germination percentage and length of germinated plantlet. Therefore it can be said that vigour index is summarization of germination and physique of the germinated seed.

- *V. mungo response to chemical treatment*

Maximum vigour index was recorded for *V. mungo* control seeds treated with 8mM $NaBH_4$ (Figure 3a, VMNABH4 vigour index, pink colour). The same concentration was showing best vigour in case of heat stressed *V. mungo* seeds (Figure 3b, VMHNABH4 vigour index, pink colour). The vigour index (%) of 8mM $NaBH_4$ treated heat stressed *V. mungo* seed was contributed by increase of average plantlet length from 19 to 27 cm (Figure 4b, VMHNABH4, red colour). We attribute the increase in vigour of *V. mungo* heat stressed seeds (treated with 8mM $NaBH_4$) to increase in average plantlet length, since the germination percentage does not differ prominently for $NaBH_4$ treatments from 1 to 8mM (Figure 3b, red colour). The same was true for controls receiving chemical treatment (Figure 1, a, red colour and Figure 4a, red colour). These observations suggest that 8mM NaBH4 treatments not only maintain the germination percentage attained during treatment with 1mM $NaBH_4$ but also stimulates the root-shoot elongation. Further, increase in concentration of $NaBH_4$ declines both germination (%) as well as average plantlet length leading to loss in vigour index (Figure 1, a, red colour and Figure 4 a, red colour).

Heat stressed *V. mungo* seeds when treated with APS, maximum vigour index (%) was recorded for

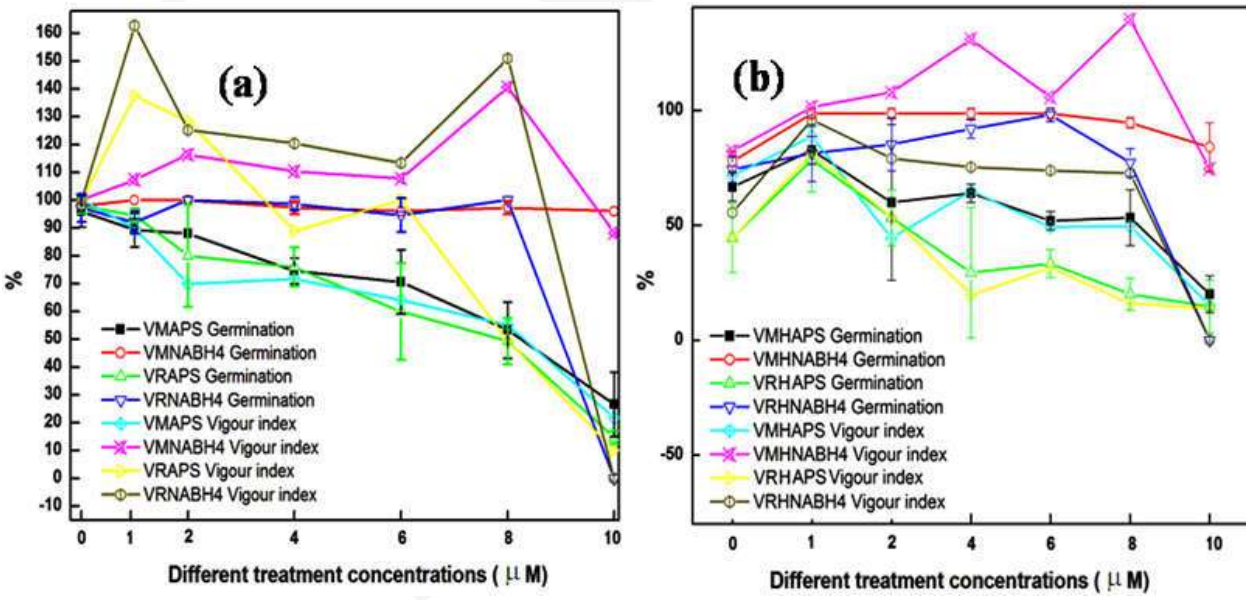

Fig. 3a: Control (without any heat stress) seeds of *V. mungo* and *V. radiata* after 7 days of germination, germination (%) and vigor index (%) data were presented with respect to different concentrations (0, 1, 2, 4, 6, 8 and 10 mM) of APS (ammonium persulphate) and $NaBH_4$ treatments on seeds. VMAPS, *V. mungo* seeds with APS treatment; VMNABH4, *V. mungo* seeds with $NaBH_4$ treatment; VRAPS, *V. radiata* seeds with APS treatment; VRNABH4, *V. radiata* seeds with $NaBH_4$ treatment.
Fig. 3b: Heat stressed seeds of *V. mungo* and *V. radiata* after 7 days of germination, germination (%) and vigor index (%) data were presented with respect to different concentrations (0, 1, 2, 4, 6, 8 and 10 mM) of APS and NaBH4 treatments on seeds. VMHAPS, heat stressed *V. mungo* seeds with APS treatment; VMHNABH4, heat stressed *V. mungo* seeds with $NaBH_4$ treatment; VRHAPS, heat stressed *V. radiata* seeds with APS treatment; VRHNABH4, heat stressed *V. radiata* seeds with $NaBH_4$ treatment.

seeds receiving 1mM treatment (Figure 3 b, VMHAPS, cyan colour). Further, treatment of seeds with increasing concentration of APS showed a decrease in the vigour index of the seeds. This decreasing germination with respect to increase in APS concentration might be attributed to decline in the percentage of germination (Figure 4 b, black colour).

The germination percentages of the plants were calculated on 7th day after sowing. If all the seeds germinate together on first day (synchronized germination) the standard deviation associated with average plantlet length will be less and *vice versa*. Therefore, the standard deviations associated with average plantlet length were also calculated as percentage (SD%). Lower the value obtained for SD (%), suggests more synchronized germination. The SD (%) for *V. mungo* optimum concentration (8mM $NaBH_4$) was lowest, suggesting that optimum vigour stimulating concentration was also synchronizing the germination (Figure 4a, pink colour). The same was also true for 1mM APS treatment (Figure 4a, yellow colour). It should be noted that unlike $NaBH_4$, APS optimum concentration treatment slightly reduce the vigour index of its control (Figure 3a, cyan colour). Summarizing all these, it can be concluded that $NaBH_4$ has a broad concentration range and safe than APS, in case of *V. mungo* seed vigour stimulation.

The optimum stimulation of vigour index was observed in case of heat stressed *V. radiata* seeds treated with 1mM $NaBH_4$ (Figure 3b, brown colour). Same concentration is also true for its controls (Figure 1, a, brown colour). With increasing concentration of $NaBH_4$ in case of heat stressed seeds, the vigour index decreases (Figure 3b, brown colour). The same was also true for control seeds except for treatment using 8mM $NaBH_4$ (Figure 1, a, brown colour). It should be noted that the vigour index of *V. radiata* control seeds treated with 1mM $NaBH_4$ was increased by 60% (Figure 3a, brown colour). The same for *V. mungo* control seeds treated with 8mM $NaBH_4$ was only 40% (Figure 3a, pink colour). This suggested that without heat stress *V. radiata* response to $NaBH_4$ is better than *V. mungo*. The 60 % increase in vigour index, due to treatment of *V. radiata* control seeds with 1mM $NaBH_4$ was due to average plantlet length increase (Figure 4a, blue colour) from 7 (without $NaBH_4$ treatment) to 12 cm (1mM $NaBH_4$). Unlike, other treatments referred above, in case of *V. radiata* seeds (control as well as heat stressed) treated with different concentrations of $NaBH_4$ the more synchronized germination is observed in seeds treated with 2mM NaBH4 rather than 1mM (Figure 3a and b, brown colour). It should also be noted in Figure 4(a and b, brown colour) the SD (%) lowest value was observed for 0.01M $NaBH_4$ treatment. It happens as per calculation because the no germination happened (Figure 3a, blue colour) and

• *V. radiata* response to chemical treatment

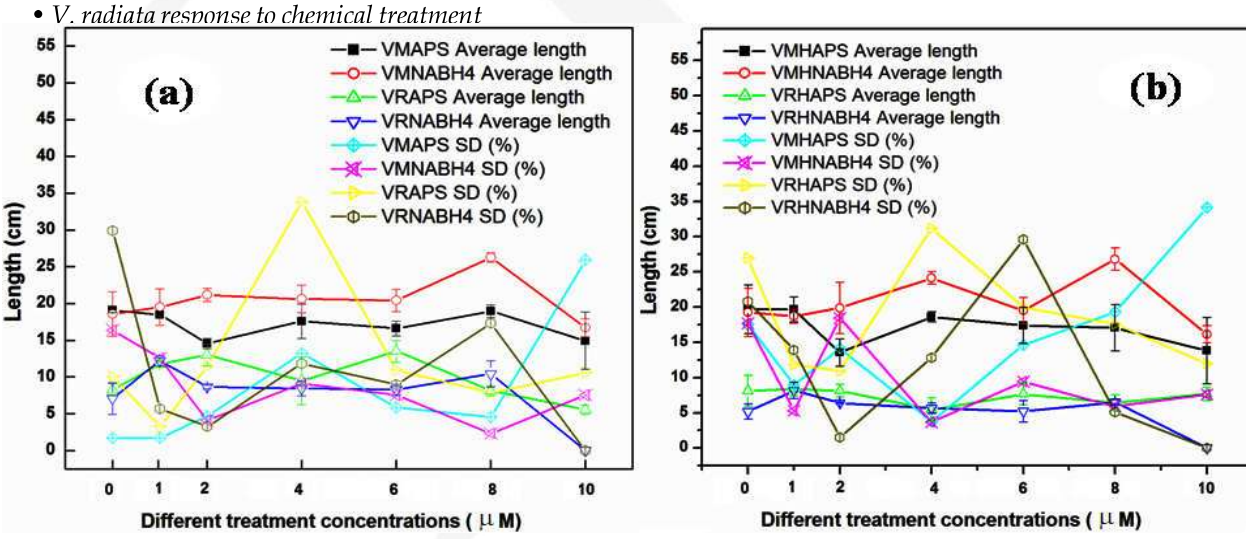

Fig. 4a: Control (without any heat stress) seeds of *V. mungo* and *V. radiata* after 7 days of germination, average plant length (cm) and SD (%) data were presented with respect to different concentrations (0, 1, 2, 4, 6, 8 and 10 mM) of APS (ammonium persulphate) and $NaBH_4$ treatments on seeds. VMAPS, *V. mungo* seeds with APS treatment; VMNABH4, *V. mungo* seeds with $NaBH_4$ treatment; VRAPS, *V. radiata* seeds with APS treatment; VRNABH4, *V. radiata* seeds with $NaBH_4$ treatment. SD (%), stands for standard deviation of length as percentage of original value.

Fig. 4b: Heat stressed seeds of *V. mungo* and *V. radiata* after 7 days of germination, germination (%) and vigor index (%) data were presented with respect to different concentrations (0, 1, 2, 4, 6, 8 and 10 mM) of APS and NaBH4 treatments on seeds. VMHAPS, heat stressed *V. mungo* seeds with APS treatment; VMHNABH4, heat stressed *V. mungo* seeds with $NaBH_4$ treatment; VRHAPS, heat stressed *V. radiata* seeds with APS treatment; VRHNABH4, heat stressed *V. radiata* seeds with $NaBH_4$ treatment. SD (%), stands for standard deviation of length as percentage of original value.

average plantlet length is zero (Figure 4a, blue colour). The same is also true for heat stressed seeds of V. radiata treated with 0.01M NaBH4 (Figure 3b and Figure 4b, blue colour).

In case of *V. radiata* seeds treated with APS, (Figure 3a and b, yellow colour) the results suggested that 1mM was the best concentration for countering the heat stressed seeds. After receiving 1mM APS treatment, the vigour index of heat stressed *V. radiata* seeds reached up to 82%, compared to 42% without APS treatment (Figure 3b, yellow colour). The same was also true for controls with 1mM APS treatment (vigour index 138%) compared to control (Figure 3a, yellow colour). Due to 1mM APS treatment in control seeds the vigour index of *V. mungo* decreases by 10% (Figure 3a, cyan colour) and the same for *V. radiata* increased by about 38% (Figure 1, a, yellow colour). Therefore, it might be suggested that *V. radiata* is more sensitive to APS and NaBH4 compared to *V. mungo*. After comparing Figure 3 (yellow colour) and Figure 4 (green colour), it can be concluded that vigour index difference among *V. radiata* seeds treated with different APS concentrations were not only because of average plantlet length difference but also due to difference in germination percentage (Figure 3a and b, green colour). The synchronized germination in case of *V. radiata* seeds treated with APS was observed for 1mM (Figure 4b, yellow colour).

Viability

The viability assays of the heat stressed seeds treated with chemicals were performed selectively (Figure 5). Only the seeds treated with optimum heat stress countering concentrations (for *V. radiata*, 1mM of both APS and $NaBH_4$; *V. mungo*, 1mM APS and 8mM $NaBH_4$) were subjected to viability assay. Figure 5 represents the viability with respect to formazan formation (represented as optical density at 480 nm) due to activity of mitochondrial dehydrogenase enzyme in seed. The optical density (OD) of *V. mungo* (about 1.0) C1 (control without heat stress and chemical treatment) decreased by about half (about 0.5) due to heat stress (C2) (Figure 5, red colour). The same trend is observed for C2 of *V. radiata*. Therefore, it suggests that 50% vigour index (Figure 3b, yellow colour) difference coincide with loss of mitochondrial dehydrogenase activity. On chemical treatment with 1mM $AgNO_3$ for 12 hr (known to stimulate dormancy by increasing abscisic acid receptor in embryo) and then 24hr APS treatment, OD decreases further below (compared to C2) to about 0.45 for both *V. mungo* (Figure 5, red colour) and *V. radiate* (Figure 3, blue colour). The same treatment when performed with $NaBH_4$ for both the seeds the OD increases to about 0.85 (Figure 5, green and black colour). This suggests that $NaBH_4$ might be a better agent to counter $AgNO_3$ induced dormancy than APS. On APS treatment the *V. mungo* seeds do not show much increase in OD (about 0.8) (Figure 5, T1, red colour)

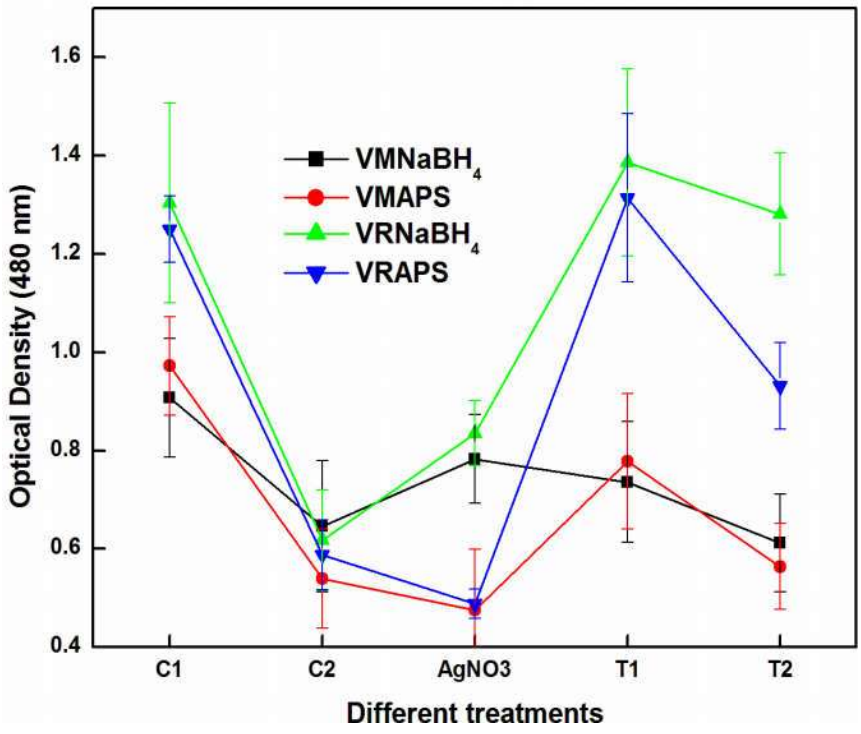

Fig. 5: Viability of chemically (APS and $NaBH_4$) stimulated seeds. $VMNaBH_4$, seeds of *V. mungo* after 8mM $NaBH_4$ treatment (except C1 and C2); VMAPS, seeds of *V. mungo* after 1 mM APS treatment (except C1 and C2); VRNaBH4, seeds of *V. radiate* after 1mM $NaBH_4$ treatment (except C1 and C2); VRAPS, seeds of *V. radiate* after 1mM APS treatment (except C1 and C2); C1, without heat stress and chemical treatment; C2, with heat stress and without chemical treatment; $AgNO_3$, 12h soaked in 1mM $AgNO_3$ solution, 24hr soaked in chemical treatments (APS and $NaBH_4$); T1, without heat stress and with chemical treatment; T2, chemical treated after heat stress.

and the T2 OD is comparable with C2. Therefore, in case of *V. mungo,* the chemical treatment did not affect the viability of the seeds tremendously (section 3.2.1.). Similarly the T1 of *V. radiata* with both chemical treatment show OD of about 1.2 compared to C1 with OD 1.3-1.4 (Figure 5, green and blue colour). On the other hand, in case of *V. radiata* T2 samples with both chemicals treatment show OD of about 0.9 (for APS treatment; Figure 5, blue colour) and 1.3 (for $NaBH_4$ treatment; Figure 5, green colour) which is significantly high compared to C2 OD (near 0.6). This coincided with our previous section result of vigour index that *V. radiata* seeds were more sensitive to the $NaBH_4$ and APS compared *to V. mungo* seeds.

Biochemical Profile of the Optimum Concentration Treated Seeds

A significant biochemical change in the seed happened with respect to germination. Out of these changes few are very important from germination prospect. Therefore to see a significant difference with respect to total protein, reducing sugar, total polyphenol and starch we analyzed only the seeds showing optimum germination due to chemical treatments along with controls. The set of samples is same as presented in viability test. The process of germination starts with imbibition of water, leading to drop of abscisic acid in embryo. Activated embryo secret gibberelic acid which activates the aleuron layer of cells to secret a-amylase; leading to hydrolysis of endosperm starch and production of reducing sugar. These soluble reducing sugars are used by the embryo for growth. The embryo and aleuron also secret minute quantities of protease and lipase depending on endosperm reserve. Therefore, increase in concentration of these chemical is a positive signal for germination in progress.

As presented in Figure 6 (black colour) the protein concentration was found highest in *V. radiata* seeds without heat stress with $NaBH_4$

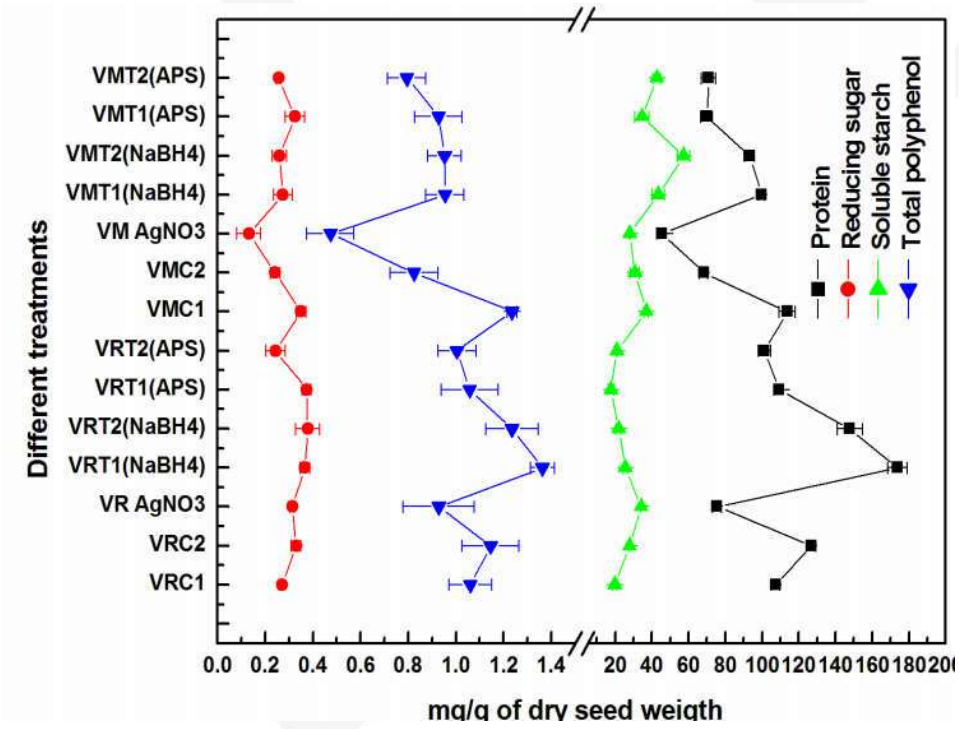

Fig. 6: Analysis of the optimum concentration chemically treated heat stressed seeds with respect to protein, reducing sugar, soluble starch and total polyphenol. VRC1, *V. radiata* seeds without heat stress and chemical treatment; VRC2, *V. radiata* seeds with heat stress and without chemical treatment; VR AgNO3, *V. radiata* seeds with 1mM $AgNO_3$ treatment for 24h; VRT1(NaBH4), *V. radiata* seeds without heat stress and with 1mM $NaBH_4$ treatment for 24 h; VRT2(NaBH4), *V. radiata* seeds with heat stress and 1mM $NaBH_4$ treatment for 24h; VRT1(APS), *V. radiata* seeds without heat stress and with 1mM APS treatment for 24h; VRT2(APS), *V. radiata* seeds with heat stress and with 1mM APS treatment for 24 h; VMC1, *V. mungo* seeds without heat stress and chemical treatment; VMC2, *V. mungo* seeds with heat stress and without chemical treatment; VM AgNO3, *V. mungo* seeds with 1mM $AgNO_3$ treatment for 24h; VMT1(NaBH4), *V. mungo* seeds without heat stress and with 8mM $NaBH_4$ treatment for 24h; VMT2(NaBH4), *V. mungo* seeds with heat stress and 01mM $NaBH_4$ treatment for 24 h; VMT1(APS), *V. mungo* seeds without heat stress and with 1mM APS treatment for 24h; VMT2(APS), *V. mungo* seeds with heat stress and with 1mM APS treatment for 24h.

treatment (VRT1 ($NaBH_4$)) followed by heat stressed seeds of *V. radiate* with $NaBH_4$ treatment (VRT2 ($NaBH_4$)). APS treated seeds of both species contain lower protein compared to $NaBH_4$. The lowest value in both *V. mungo* and *V. radiata* series is seen when treated with $AgNO_3$ only. These seeds were never germinated. The overall patter of protein content coincides with the vigor index pattern. As presented in Figure 6 the protein and polyphenol content varies several folds among germinated and non germinating seeds ($AgNO_3$ treated). Similar fold of variation is not observed for soluble starch and reducing sugar (Figure 6, red and green colour). Soluble proteins might be constant in all seeds, but once germination start the a-amylase production and cell division of embryo might leads to increase in protein content. Similarly, polyphenols content is also found to highly sensitive to germination process. Soluble starch may not change that contrastingly. Reducing sugar level might not be changing as the reducing sugars are utilized by embryo for growth and development but it follows the pattern of increasing and decreasing germination with exception in VRC2 (Figure 6, red colour).

V. radiata seed germination has been studied by several researchers in recent years [22,23,24]. Metal ion toxicity like Cd and Fe deficiency are reported for *V. radiata* [25]. The role of aleuron layer redox condition with respect to germination is reported by Saleh and Kebeish [26]. They also suggested that during germination aleuron layer cells undergo programme cell death due to production of huge amount of reactive oxygen species (ROS) [26]. APS might be stimulating the germination promoting the same by supplying exogenous ROS. Further experimentation is needed to establish the fact.

Comparing the treatment on two different seeds it can be concluded that $NaBH_4$ more effective for vigour stimulation of *V. radiata* compared to APS. APS is more effective on *V. mungo* than $NaBH_4$. Non optimal concentrations of APS (Figure 3a, black and green colour) are found to decrease germination percentage in both seeds, whereas the same of $NaBH_4$ have less detrimental effect on germination (Figure 3a, red and blue colour), except for 0.01M $NaBH_4$ treatment to *V. radiata* seeds.

From the biochemical analysis it can be concluded that soluble protein and total polyphenol content might be taken as marker for germination (Figure 6, black and blue colour respectively). The present study successfully able to stimulate increase the germination process of *V. mungo* and *V. radiata* seeds using $NaBH_4$ and APS and in future further study might be performed for looking into the productivity of the plants.

The 1mM APS concentration treatment to control *V. mungo* seed decreases the vigour (Figure 3a, cyan colour). Therefore, 1mM APS might be detrimental for *V. mungo* seeds, which does not received any heat stress. The present research opens our eye regarding plant seed treatment and suggests that there are huge possibilities of studying different toxic chemicals in low doses on many different species for other beneficial effects.

Hormones as seed germinator stimulator is reviewed by Miransari and Smith (2014) [27]. Non-hormone seed germination stimulators like reactive oxygen species (ROS) [28,29]; light [30] and testa rupture [31].

Conclusion

We hope that *V. mungo* and *V. radiata* cultivating farmers will get benefited by this technology and our recommendation are for *V. mungo* (black gram) $NaBH_4$ (8mM) or APS (1mM) should be used for soaking seeds for 24 h. Similarly for *V. radiata* (green gram) $NaBH_4$ (1mM) or APS (1mM) might be used. For getting synchronized germination in the field the above concentration treatment will work fine except for *V. radiata* (green gram) $NaBH_4$ stimulation concentration 2mM might give better result than 1mM. Future, investigation to evaluate mechanism is necessary to boost the simulation further. It is pertinent to note that the present work is aimed at demonstrating the role of $NaBH_4$ and APS as stimulator for seed germination. We are not excluding the possibility that silver nanoparticle might also stimulate germination, which will be a different study altogether.

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Competing Financial Interests

The authors declare no competing financial interests.

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