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Enhancement the Seedling Quality OF Radish through Priming with H_2O_2

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Abstract: *A study with three independent experiments for the seeds of three vegetable species was set at the roof top of Sunway Dormitory near the Bus Terminal, Dinajpur, Bangladesh. The aims were to evaluate the physiological fluctuations in the seeds of those three vegetable seeds primed with the aqueous solutions of H_2O_2 and to find the suitable strength(s) of H_2O_2 as the priming material for their highest seedling quality. The seeds of radish cv. Rake were the testing materials.* ***Each*** *experiment had eight treatments: T_1 (control/no priming), T_2 (hydro-priming in plain H_2O) and other six aquatic solutions of H_2O_2: T_3 (0.5), T_4 (1), T_5 (1.5), T_6 (2), T_7 (2.5) and T_8 (3%). The seeds were soaked for six hours in those seven media (T_2 toT_8). The seeds were dibbled in wooden seed flats filled-in with coarse sand. The experiments were laid-out in the Randomized Complete Block Design with three replications. Data collected for the 13 traits were: % germination, % abnormal seedlings, shoot length, root length, seedling length, shoot dry matter, root dry matter, seedling dry matter, number of secondary roots (>1cm) per seedling, number of true leaves per seedling, relative growth rate, seedling vigor index, and root : shoot ratio (dry weight basis). Except the first two traits, the rest 11 were collected at three stages: 10, 20 and 30 days after dibbling (DAD). It was lucid that H_2O_2 was significantly ($P\leq0.05\%$) effective to improve most of the traits noted. But 1.5% concentration was utmost helpful for for radish seeds were toxic and hindered the maximum parameters for all the three species. Nevertheless, further studies with different varieties of those three vegetables species with variable doses of H_2O_2, priming time and temperature could be explored before drawing valid conclusions.*

1. Introduction

Radish (Raphanus sativus), a member of the family Cruciferae, is a popular vegetable in both tropical and temperate regions of the world (Becker, 1962). Radish is an important root vegetable crop widely cultivated for its tender roots as well as succulent foliage and immature pods (used largely in salad and for culinary purposes). Fresh root provides just 16 calories per 100 g, nonetheless, they are a very good source of anti-oxidants, electrolytes, minerals, vitamins and dietary fiber. Fresh roots are rich in vitamin C and provide about 15 mg of vitamin C per 100 g. In addition, they contain adequate levels of folates, vitamin B-6, riboflavin, thiamin, and minerals, such as iron, magnesium, copper, and calcium (Urbano, 2012).The characteristic pungentflavor of radish is due to is othiocyanate (Kushwah, 2016). It is used for neurological headaches, chronic diarrhea, urinary complaints, sleeplessness and piles (Singh and Bhandari, 2015).

Seed germination is one of the vital stages in the life cycle of seeded plants. Germination is a very complex process starting with the imbibition of H_2O and involves events related with the transition of a dry quiescent and/or dormant seed to the metabolically active state (Kranner*et al*., 2011, and Schopfer*et al*., 2001). The emergence of the embryonic axis through structures surrounding the embryo is the final stage of germination (Weitbrecht*et al*., 2011). In this link, seed priming is used as a means to enhance seed performance, notably in terms to the rate and the uniformity of germination

(Taylor *et al.*, 1998). Seed priming is known as the pre-sowing approach to govern seed germination and seedling development by modulating pre-germination metabolic activities prior to emergence of the radicle and usually enhances germination rate and plant growth (Bradford, 1986).Various physiological and bio-chemical changes happen in seeds during priming as a result of osmotic conditioning. A wide range of pre-sowing hydration techniques is used to enhance seed germination responses. These include equilibrium under conditions of high humidity (Finnerty *et al.*, 1992), soaking in plain H_2O (Coolbear and McGill, 1990) or osmotic solutions (Knypl and Khan, 1981) and having equilibrium with a matric potential controlling surface (Hardegree and Emmerich, 1992). Hydro-priming, osmo-priming (with mannitol or PEG 6000) and halo-priming (with KCl, KNO_3 or calcium salts) are effective for seedling establishment under harsh conditions (Toselli and Casenav, 2003). The priming enhances rapid and uniform emergence, high vigor and better yield, which has practical utilities, preferably under water stress conditions (Black *et al.*, 2006).

H_2O_2 (Hydrogen peroxide; an easily available cheap safe chemical oxidant) is a reactive molecule playing crucial roles in plants, especially under unfavorable germination conditions, developmental processes and in resisting stresses in reactive oxygen species/ROS (El-Maarouf-Bouteau and Bailly, 2008). H_2O_2 acts as a signaling molecule in the beginning of seed germination involving specific changes at the proteomic, transcriptomic and hormonal levels (Afghani and Taheri, 2012, and Demir *et al.*, 2012). Priming of seeds with H_2O_2 leads to break primary dormancy (Jann and Amen, 1997); secondary dormancy provoked by salinity (Jia *et al.*, 2002) and germination inhibitors (Ogawa and Masaki, 2001). It acts as a stress signal in plants and hence exogenous uses of H_2O_2 in the right dose ameliorates seed germination, reduces time to germinate and seedling growth in many crops (Patade*et al.*, 2012). So, it has special roles, especially in invigorating seeds with low vigor including long-term stored seeds in gene banks.The objective of the study was to evaluate the physiological changes of the seedlings of radish primed with $H_2O_2$and find out the best concentration.

2. MATERIALS AND METHODS

The present research work was conducted at the roof top of Sunway Dormitory near the Bus Terminal, Dinajpur, Bangladesh. The seeds of, radish cv. Rake was used as the testing seed materials (Plates 1 – 3).

2.1. Experimental Treatments

This single factor experiment was designed with eight treatments viz. T_1 (control/no priming), T_2 (hydro-priming in plain H_2O) and other six aquatic solutions of H_2O_2 viz.T_3 (0.5%), T_4 (1.0%), T_5 (1.5%), T_6 (2.0%), T_7 (2.5%) and T_8 (3.0%). This single factor experiment was set in the Randomized Complete Block Design (RCBD) with three replications.

2.2. Seed Flats and their Arrangements

The seed were debbled in wooden seed flats. The size of each flat was 50×50×15cm. Firstly; the flats were set on the roof top. Then blue polyethylene sheet was spread at the bottom of the flats to protect washing away of sand from the seed flats. Then flats were filled-in with coarse sand.

2.3. Preparation of the Required H_2O_2 Solutions

The required solutions were prepared by diluting the required amounts of the H_2O_2 (30% strength, Plate 1) with H_2O to get these six concentrations: 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0%.

Plate1. *The container of the H_2O_2(30%)used in the study*

2.4. Priming Process

At first, only H_2O and those six solutions were taken in plastic glasses separately. The glasses were marked about the treatments and replications with a permanent glass marker. Then the 200 seeds for each replication were taken in plastic glasses to soak in the desired solutions for six hours in the Laboratory of Horticulture (Plate 2).

Plate2. *Priming process with H_2O_2*

2.5. Dibbling the Seeds and Caring the Seedlings

The unprimed and the primed seeds were then dibbling immediately in the seed flats (Plate 3) at the depth of 2cm in lines on the 10th March, 2019 at the distance of 5cm between rows and seeds too. After sowing, the seeds were covered with hyaline polyethylene sheet and concrete poles (at one foot high) to protect the seeds and seedlings from heavy rainfall. Light watering with a watering cane was done as needed. Hand weeding was also done as per need.

Plate3. *Growing the seedlings in the seed flats*

2.6 Data Collection

The data were collected for % germination and % abnormal seedlings at 10 days of dibbling and shoot length, root length, seedling length, shoot dry matter, root dry matter, seedling dry matter, number of secondary roots (>1cm) per seedling, number of true leaves per seedling, relative growth rate, seedling vigor index, and root: shoot ratio (dry weight basis) were at 10, 20 and 30 DAD.

Germination (%) and normal seedling (%) were observed and counted as per the ISTA (2010) rules daily up to 10 DAD. For dry matter the normal seedlings were cut and divided into roots and shoots with a razor blade from each treatment and replication wise. Then those were first sundried separately for two days. After that, those were dried at 80°C for 48 hours in an electric oven (Memmrert, ULP 400). Then the dry weights of shoots were recorded up to four decimal places with an electric digital balance (Ohaus, pioneer pro PA214). Finally, the dry weights were expressed in gram per 100-seedling basis. Those processes were repeated with the normal seedlings only obtained from the 10, 20 and 30 DAD. Relative growth rate (RGR)was calculated as per Williams (1946) formula and Seedling vigor index (SVI) was calculated as per Orchard (1977) and Baki and Anderson (1973) viz. SVI = Mean seedling (root + shoot) length (MSL) in cm ×% germination (PG)

2.7. Statistical Analyses

The analyses of variances (ANOVA) were done and the means were separated using Duncan's Multiple Range Test (DMRT). The MSTAT-C Statistical Package program was used for it.

3. RESULTS AND DISCUSSION

3.1. Germination %

Seed priming with different H_2O_2 treatments significantly ($P \leq 0.05$) affected the % germination of bitter gourd (Fig. 1). The germination % decreased significantly with the increasing in H_2O_2 concentrations. The highest germination % was recorded in T_5 (91.0) but the lowest % was recorded in T_8 (80.67%).The priming of seeds with H_2O_2 changes the germination mechanism due to improved breaking of primary and secondary dormancies and prevented suffocation. In this experiment, the highest % germination was found in seeds primed with 1.5% H_2O_2. But Iqbal *et al.* (2001) got better germination in okra seeds at 2% H_2O_2. Again, the higher doses (>1.5%) of H_2O_2 probably caused toxic effects on the germination and so, the % germination became low, even than the unprimed and the hydro-primed seeds. The H_2O_2 levels >2% resulted in severe injury to germinating seeds (John and Duval, 2000). Mustafa (2017) studied cucumber, swamp cabbage, radish and Indian spinach seeds and had better germination at 1% H_2O_2. While working with bitter gourd and bottle gourd, Lima (2017) found better germination at 1.5% H_2O_2. But Iqbal *et al.* (2001) found higher germination in okra seeds at 2% concentration with H_2O_2. Furthermore, Kaya *et al*. (2006) reported that priming increased % germination of sunflower seeds under drought stress.

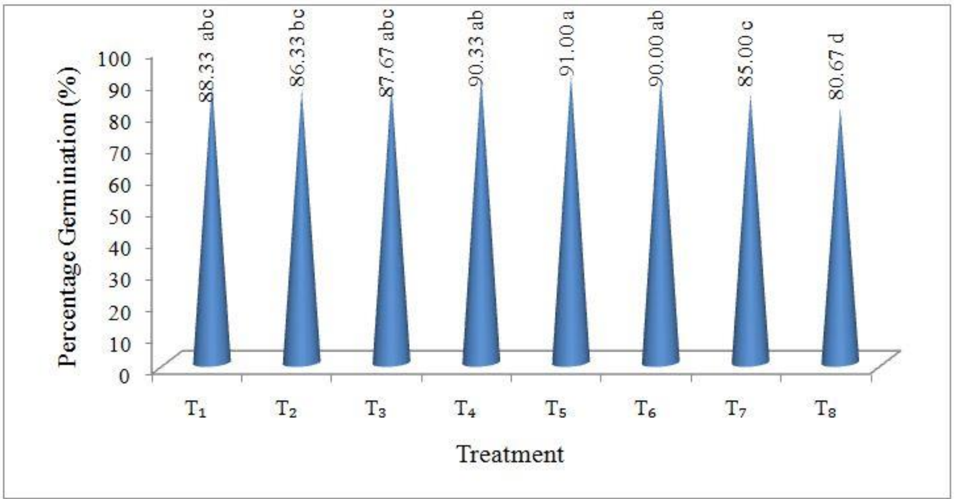

Fig1. *Effect of priming with H_2O_2 on the % germination of radish*

3.2. Abnormal Seedling (%)

There was significant ($P \leq 0.05$) difference among the treatments in respect of the percentage of abnormal seedlings (Fig. 2). The result revealed that during the germination period, the % abnormal seedlings increased gradually with the increased in H_2O_2 concentrations. The maximum % of abnormal seedlings was observed in T_8(5.66) but the minimum was in T_5 (2.00%).The results showed that the occurrence of abnormal seedlings increased with the increasing doses of H_2O_2. In bitter gourd and bottle gourd seeds, Lima (2017) noted the minimum% abnormal seedlings with 1.5% while the maximum at the 3% H_2O_2. Parallely, Mustafa (2017) recorded the minimum % abnormal seedlings with swamp cabbage, radish and Indian spinach seeds at 1% but the maximum abnormal seedlings with 3% H_2O_2.Furthermore, Kaya *et al*. (2006) reported that priming reduced the number of abnormal seedlings of sunflower seeds under drought stress.

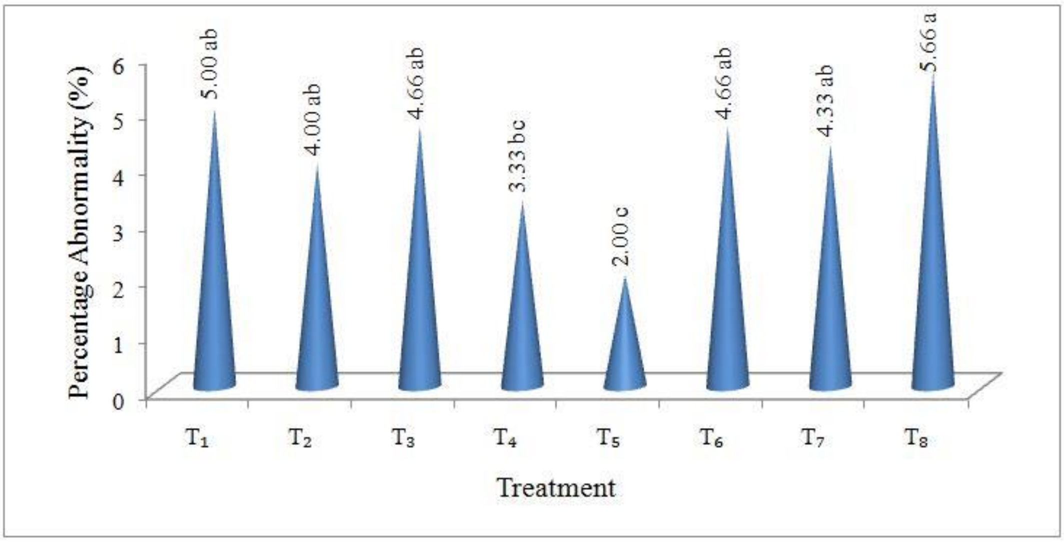

Fig2. *Effect of priming with H_2O_2 on the % abnormal seedlings of radish*

3.3. Shoot, Root and Seedling Length (cm)

There was significant ($P \leq 0.05$) variation in shoot length among the treatments at all the three DADs (Table 1). The highest shoot length at the 10 and 30, 20 DAD was found in T_5 (6.12, 8.03 and 10.29 cm) while the lowest was in T_8 (4.53, 5.39 and 7.85cm). At 10, 20 and 30 DAD, the maximum root length was found inT_5 (4.27, 11.19 and 12.45 cm) and the minimum amount was in T_8 (3.23, 7.82 and 9.30cm). In seedling length, at the 10, 20 and 30 DAD, the maximum length was recorded in T_5 (10.40, 19.23 and 22.59) while the minimum was found in T_8 (7.77, 13.39 and 17.17 cm). In addition, the higher doses (>1.5%) of H_2O_2 probably caused deleterious effects on the shoot length and so, it became shorter, even than the unprimed and the hydro-primed seeds. However, the applications of H_2O_2 at doses of 1.8mM each eight days broccoli seedlings, increased the stem length and fresh weight, whereas the dose 1.4mM increased the biomass of broccoli seedlings (León-Vargas *et al*., 2016). In bitter gourd and bottle gourd seeds, Lima (2017) measured the minimumshoot, root and seedling length with 1.5% whiles the maximum at the 3% H_2O_2. Mustafa (2017) also noted the minimum shoot, root and seedling length with swampcabbage, radish and Indian spinach seeds at 1% but the maximum shoot length with 3% H_2O_2. But 1.0% was very helpful for country bean and yard long bean while 1.5% for bottle gourd and bitter gourd seeds. Above 1.5%, others were toxic (Lima, 2017).The influence of seed priming in improving the root length had been well noticed in ground nut by Rahman*et al*. (1997).Nandi *et al*. (2017) noted the maximum root length in seedlings of chilli raised from the seeds treated with the 1.0% H_2O_2.

Table1. *Effect of priming seed with H_2O_2 on shoot, root and seedling length in okra*

Treatments	Shoot length (cm)			Root length (cm)			Seedling length (cm)		
	10 DAD	20 DAD	30 DAD	10 DAD	20 DAD	30 DAD	10 DAD	20 DAD	30 DAD
T_1	5.04b	6.15b	8.41b-d	3.56bc	8.35cd	9.78cd	8.61bc	14.50d	18.20de
T_2	5.25b	7.43a	9.03b	3.69bc	8.82c	11.18b	8.94b	16.26bc	20.21b
T_3	5.08b	7.37a	8.91bc	3.63bc	9.14bc	11.14b	8.71b	16.53b	20.04bc
T_4	6.03a	7.18a	9.12b	4.01ab	9.96b	12.05a	10.04a	17.15b	21.14b
T_5	6.12a	8.03a	10.29a	4.27a	11.19a	12.45a	10.40a	19.23a	22.59a
T_6	5.28b	5.95b	8.14cd	3.84ab	8.84c	10.42bc	9.12b	14.80d	18.57d
T_7	5.04b	6.09b	8.02d	3.71bc	9.23bc	10.94b	8.75b	15.32cd	18.96cd
T_8	4.53b	5.39b	7.85d	3.23c	7.82d	9.30d	7.77c	13.39e	17.17e
LSD $_{(0.05)}$	0.724	0.899	0.767	0.453	0.886	0.747	0.863	1.05	1.18
CV %	7.80	7.66	5.02	6.91	5.51	3.91	5.45	3.81	3.46

The figures with different letters differ among themselves at the 5% level of probability.

T_1 = Control/no priming, T_2 =Hydro-priming in plain H_2O), T_3 = 0.5% aquatic solutions of H_2O_2, T_4 = 1.0% aquatic solutions of H_2O_2, T_5 = 1.5% aquatic solutions of H_2O_2, T_6 = 2.0% aquatic solutions of H_2O_2, T_7 = 2.5% aquatic solutions of H_2O_2 and T_8 = 3.0% aquatic solutions of H_2O_2

3.4. Shoot, Root and Seedling Dry Matter (g per 100-Seedling Basis)

There was significant ($P \leq 0.05$) variation in shoot dry matter at all the three DADs(Table 2). The maximum dry matter of shoots at 10, 20 and 30 DAD, was observed in T_5 (8.48, 16.66 and 32.97 g) while the minimum was in T_8 (5.86, 11.03 and 19.01g). At 10, 20 and 30 DAD the highest dry matter of roots was found in the T_5 (0.866, 2.12 and 5.66) and the lowest was recorded from T_8 (0.556, 1.15 and 2.84g). The highest dry matter accumulation in seedlings at the 10, 20 and 30 DAD were in T_5 (9.33, 18.78 and 38.64 g) while the lowest was observed in T_8 (6.50, 12.18 and 21.85 g). In bitter gourd and bottle gourd seeds, Lima (2017) experienced the maximum shoot, root and seedling dry matter primed with the 1.5% H_2O_2. It is vital to note that, the higher doses (>1.5%) of H_2O_2 probably caused deleterious effects on the shoot dry matter and so, it became less, even than the unprimed and the hydro-primed seeds. However, the applications of H_2O_2 at doses of 1.8mM each eight days broccoli seedlings, increased the stem length and fresh weight, whereas the dose 1.4mM increased the biomass of seedlings (León-Vargas *et al*., 2016).. Furthermore, Kaya*et al*. (2006) reported that priming increased seedling dry weight of sunflower under drought stress.

Table2. *Effect of priming seed with H_2O_2 on shoot, root and seedling dry matter of okra*

Treatments	Shoot dry matter			Root dry matter			Seedling dry matter		
	10 DAD	20 DAD	30 DAD	10 DAD	20 DAD	30 DAD	10 DAD	20 DAD	30 DAD
T_1	6.54cd	13.92b	25.64bc	0.676ab	1.39b	3.79c	7.25c	15.31c	29.44b
T_2	7.09bc	13.65b	25.77bc	0.730ab	1.30b	5.21ab	7.73bc	14.95c	30.99b
T_3	6.51cd	13.80b	22.08cd	0.648ab	1.33b	3.18de	7.16c	15.13c	25.26cd
T_4	7.84ab	15.93a	22.65b-d	0.813ab	1.51b	4.82b	8.60ab	17.44b	27.47bc
T_5	8.48a	16.66a	32.97a	0.866a	2.12a	5.66a	9.33a	18.78a	38.64a
T_6	6.86bcd	13.64b	19.66d	0.540b	1.32b	3.64cd	7.58bc	14.83c	23.40d
T_7	6.38cd	12.16c	26.06b	0.670ab	1.17b	3.79c	7.11c	13.39d	29.86b
T_8	5.86d	11.03c	19.01d	0.556b	1.15b	2.84e	6.50c	12.18d	21.85d
$LSD_{(0.05)}$	1.084	1.201	3.469	0.2538	0.4069	0.5340	1.128	1.313	3.622
CV %	8.91	4.95	8.18	21.03	16.37	7.40	8.41	4.91	7.29

The figures with different letters differ among themselves at the 5% level of probability.

T_1 = Control/no priming, T_2 =Hydro-priming in plain H_2O), T_3 = 0.5% aquatic solutions of H_2O_2, T_4 = 1.0% aquatic solutions of H_2O_2, T_5 = 1.5% aquatic solutions of H_2O_2, T_6 = 2.0% aquatic solutions of H_2O_2, T_7 = 2.5% aquatic solutions of H_2O_2 and T_8 = 3.0% aquatic solutions of H_2O_2

3.5. Number of Secondary Roots and True Leaves Per Seedling

Number of secondary roots and true leaves per seedlings varied significantly ($P \leq 0.05$) among the treatments at all the three DADs (Table 3).At the 10, 20 and 30DAD, the maximum number of secondary roots was in T_5 (8.10, 11.11 and 20.89) whereas the minimum number was in T_8 (4.90, 9.67 and 17.07). The maximum number of true leaves at 10, 20 and 30 DAD were in T_5 (2.37, 4.58 and 5.86) while the minimum was in T_8 (2.10, 3.59 and 4.36). Similar types of result were also reported by Lima (2017) and Mustafa (2017). The excessive accumulation of H_2O_2 leads to cellular oxidative damage and even programmed death and thus becomes poisonous for seedlings (Levine *et al*., 1994 and Prasad *et al*., 1994).The concentrations of H_2O_2 came up to 5mM, it played an opposite role to inhibit the growth of adventitious roots and seriously damaged those found by Deng *et al*., 2012.

Table3. *Effect of priming seed with H_2O_2 on number of secondary root and true leaves of okra*

Treatments	Number of secondary roots			Number of true leaves		
	10 DAD	20 DAD	30 DAD	10 DAD	20 DAD	30 DAD
T_1	5.90cd	10.29bc	18.63c	2.28a	3.66 b	5.21c
T_2	6.72bc	10.64ab	18.63c	2.20a	3.94b	5.30bc
T_3	7.20ab	9.910bc	17.84cd	2.28a	3.83b	5.58ab

T_4	7.06ab	10.45ab	19.96b	2.20a	3.60b	5.30bc
T_5	8.10a	11.11a	20.89a	2.37a	4.58a	5.86a
T_6	7.45ab	10.11bc	17.76cd	2.28a	3.63b	5.26bc
T_7	5.59d	10.10bc	17.57d	2.25a	3.71b	5.00c
T_8	4.90d	9.67c	17.07d	2.10a	3.59b	4.36d
LSD $_{(0.05)}$	1.071	0.662	0.839	0.259	0.383	0.318
CV %	9.23	3.68	2.59	6.58	5.71	3.47

The figures with different letters differ among themselves at the 5% level of probability.

T_1 = Control/no priming, T_2 =Hydro-priming in plain H_2O), T_3 = 0.5% aquatic solutions of H_2O_2, T_4 = 1.0% aquatic solutions of H_2O_2, T_5 = 1.5% aquatic solutions of H_2O_2, T_6 = 2.0% aquatic solutions of H_2O_2, T_7 = 2.5% aquatic solutions of H_2O_2 and T_8 = 3.0% aquatic solutions of H_2O_2

3.6. Seedling Vigor Index (SVI)

There was significant variation ($P \leq 0.05$) in seedling vigor index among the treatments judged at all the three DADs (Table 4). At the 10, 20 and 30 DAD, the topmost seedling vigor index was noted in T_5 (919.4, 1749 and 2056) while the minimum value was in T_8 (626.7, 1080 and 1409). Lima (2017) experienced the maximum seedling vigor index at the 1% H_2O_2.Mustafa (2017) also recorded the maximum seedling vigor index of swamp cabbage, radish and Indian spinach seeds. Similar types of results were also reported by Nandi *et al.* (2017) in chili seeds.

Table4. *Effect of seed priming with H_2O_2 on Seedling vigor index, relative growth rate and root shoot ratio of okra*

Treatments	Seedling vigor index			Relative growth rate		Root shoot ratio		
	10 DAD	20 DAD	30 DAD	10-20 DAD	20-30 DAD	10 DAD	20 DAD	30 DAD
T_1	753.8b	1281e	1608d	0.070a	0.066a	0.096a	0.093a	0.146b
T_2	772.4b	1404cd	1747c	0.060a	0.073a	0.100a	0.090a	0.196ab
T_3	764.1b	1449c	1757c	0.070a	0.053a	0.096a	0.093a	0.136b
T_4	906.9a	1549b	1910b	0.066a	0.043a	0.100a	0.090a	0.210a
T_5	919.4a	1749a	2056a	0.066a	0.090a	0.096a	0.123a	0.166ab
T_6	821.1b	1332de	1672cd	0.063a	0.043a	0.103a	0.106a	0.180ab
T_7	743.8b	1302e	1612d	0.056a	0.070a	0.100a	0.093a	0.140b
T_8	626.7c	1080f	1409e	0.056a	0.050a	0.090a	0.090a	0.143b
LSD $_{(0.05)}$	78.10	93.35	102.4	0.055	0.055	0.055	0.05538	0.055
CV %	5.66	3.83	3.40	19.48	14.53	11.77	11.74	11.26

The figures with different letters differ among themselves at the 5% level of probability.

T_1 = Control/no priming, T_2 =Hydro-priming in plain H_2O), T_3 = 0.5% aquatic solutions of H_2O_2, T_4 = 1.0% aquatic solutions of H_2O_2, T_5 = 1.5% aquatic solutions of H_2O_2, T_6 = 2.0% aquatic solutions of H_2O_2, T_7 = 2.5% aquatic solutions of H_2O_2 and T_8 = 3.0% aquatic solutions of H_2O_2

3.7. Relative Growth Rate (RGR)

There was insignificant difference for the relative growth rate among the treatments compared (Table 3). At 30 DAD, the maximum relative growth rate was recorded in T_5 (0.090) the lowest was found in $T_4$and T_6 (0.043).Mustafa (2017) found highest relative growth rate at 3% (0.22)and Lima (2017) recorded relative growth rate in control/no priming (0.11).

3.8. Root Shoot Ratio (Dry Weight Basis)

There was significant difference for the root shoot ratio among the treatments at the 20 and 30 DAD but non-significant at 10 DAD (Table 4). At 20 and 30 DAD, the topmost root shoot ratio was noted in T_4(0.210) while the minimum was found in T_3(0.136).Lima (2017) noted the maximum root: shoot ratio (0.34) in seedlings of yard long been raised from the seeds treated with the 1% H_2O_2 and minimum was 3% H_2O_2.

4. CONCLUSIONS

So, from the results, it can be concluded thatH_2O_2had optimistic effects on the seedling qualities. Among the six concentrations of the H_2O_2, 1.5% was suitable for radish seeds. Above those concentrations, others were somewhat toxic as those hindered a lot of the parameters evaluated.

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