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Effect of sodium perchlorate treatment on germination of guar

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Abstract
The present study aimed at assesses the effect different concentration of chlorate in breaking dormancy of Four Varieties of *Cyamopsis tetragonoloba* seeds. The four varities of seeds (RGC 936, RGC 1002, RGC 1003 and RGC 1017) was collected from Agriculture Research Centre Durgapura, Jaipur, Rajasthan. Seeds were again treated using different concentrations of Clorox (NaOCl) i.e 0.5 %, 1%, 2%, 3% and 4% for 12 hours. A total of 10 seeds of each variety were placed in 90-mm-diameter Petri dishes on 2 layer of Watman No. 1 filter paper soaked with sterile distilled water and untreated seeds of each variety were grown in sterile distilled water as control. In comparison to control treated seeds were observed for germination percentage, radical length, plumule length, seedling length, seedling vigour index (SVI), fresh weight and dry weight. The results of the present study, thus, indicate the treatment of seeds with 4% NaOCl is most effective in seed germination although one type of seed remained dormant in the experiment which shows that some other method for breaking its dormancy is required for further study.

Keywords: sodium, guar, germination, *Cyamopsis tetragonoloba*, seeds

Introduction
Cyamopsis tetragonoloba (L) Taub commonly known as Guar, it belongs to family Fabaceae. Guar is a small drought resistant herb and commonly cultivated in India and Pakistan, in India particularly at Rajasthan, Punjab and Haryana [1]. Guar is a self pollinated diploid annual legume cultivated during the summer season. Guar flowers are purple to pink, zygomorphic, approximately 8 mm long. The calyx has five unequal linear teeth. The standard is orbicular, and the wing petals are oblong, while the keel petals are as long and broad as the wings. The ten stamens are all fertile, and the filaments form a tube while the anthers are aciculate, pollen grains are circular and 40-43μm in diameter. The stigma expands into a head-shape while, the style is short and slender [2]. The Gum obtained from the endosperm of the seed is used for medicinal purpose. Many process like hydraulic fracturing (fracking) extraction of oil and shale gas increased through the use of guar gum therefore the demand for the plant has increased substantially [3]. In arid and semiarid climate this plant is used as food and treatment of various ailments. In fact, agriculturists in Rajasthan follow crop-rotation and use guar as a source to replenish the soil with essential fertilizers and nitrogen fixation, before the next crop [4].

Seed germination is the initial step in the plant life cycle and the ability of seed for imbibition is the vital regulatory step in development of plants [5]. Currently seed priming is one of the widely used method for increasing the rate of seed germination, in priming, seeds are kept in contact with limited water under controlled conditions which provide condition for some of physiological processes of germination to occur. It also allows the accumulation of certain oxidant compounds used for activating special enzymes that ultimately break dormancy and allows increasing germination speed [6].

Seeds of most arid and semi-arid plants are not able to germinate promptly although subjected to favourable condition for germination due to water impermeable seed coat. Due to this seeds of such species are required to be subjected for some physical or chemical treatment to break dormancy and obtain uniform germination [7-8]. Various mechanical or chemical treatment have already been reported for seed germination and treatment of seed with NaOCl is found effective in accelerating seed germination [9]. The aim of this paper is to study the effect of NaOCl treatments on seed germination and vigor.

Materials and Methods

Collection of plant material

Seeds of Guar (*Cyamopsis tetragonoloba* (L) Taub) var. RGC 936, RGC 1002, RGC 1003 and RGC 1017 were collected from Agriculture Research Centre Durgapura, Jaipur, Rajasthan.

Surface sterilization of seeds

The study was carried out at the Department of Botany, University of Rajasthan, Jaipur. Seeds were superficially sterilized with 70 % ethanol for 1 min. and then rinsed with distilled water 3 times each for 5 min, and then dried with paper towel. Seeds were again treated using different concentrations of Clorox (NaOCl) i.e 0.5 %, 1%, 2%, 3% and4% for 12 hours. At last, it was rinsed five times with sterile distilled water before allowing germination.

Experiment for seed germination

A total of 10 seeds of each variety were placed in 90-mm-diameter Petri dishes on 2 layer of Watman No. 1 filter paper soaked with sterile distilled water and untreated seeds of each variety are also grown in sterile distilled water as control. These petri dishes containing seeds were kept at room temperature (25°C ± 1°C) under normal light for germination. Four replicates were used for each variety plus control. The number of germinated seeds was counted daily for 8 days after which no further seed germination occurred. The appearance of 2 mm or more of radicle length was considered as germination.

$$Germination = \frac{Nd}{N} \times 100$$

where Nd: number of germinated seed till that day and N: Total number of seeds.

The seedling vigor index (SVI) was calculated according to following formula (Abdul-Baki and Anderson, 1970)

$$SVI = (\text{seedling length} \times \text{germination percent})/100$$

Statistical analysis: In our experiment we used Two way ANOVA analysis by using GraphPad Prisim 5.01 software and considered P <0.05 as statistically significant value.

Result

Germination percent

Result showed that RGC 936 and RGC 1003 varieties of seeds of *Cyamopsis tetragonoloba* are more responsive towards NaOCl treatment. These two varieties showed germination at almost all concentration. RGC 1002 seeds remain dormant at all concentration. The experiment showed that the best concentration for germination of seeds of *Cyamopsis tetragonoloba* is 4% (Table 1).

Radicle and Plumule length

In table 2, length of radical and plumule have been shown. Four varieties of seeds treated with different concentration followed same trend, as the concentration of NaOCl increases the length of radical and plumule also increased. The length and weight of all varieties of seeds remained very less in control (untreated) on 8th day but at concentration of 4% all varieties showed increase in the length of radical and plumule on 8th day.

Seedling length and Seed vigor

Seedling length of RGC 936, RGC1003 and RGC 1017 varieties on 8th day was 5.26±0.76, 3.73±0.69 and 6.06±0.68 respectively. The maximum seedling length was shown by RGC1017 seeds treated with 4% of NaOCl. Seedling vigor increased for seeds treated with increasing concentration of NaOCl. Seedling vigor for untreated and seeds treated with 0.5% of NaOCl was very less. The highest seedling vigour was observed for RGC 936 i.e 4.20.

Fresh and dry weight

As shown in Table 2, the maximum fresh weight of seedling was observed for plants treated with 4 % NaOCl i.e. 0.12±0.02, 0.18±0.06 and 0.11±0.03 gm/plant of RGC 936, RGC1003 and RGC 1017 respectively. Untreated, Treated with 0.5% NaOCl showed less weight and RGC1002 seeds remain dormant therefore no weight was masured for this variety of seed. The result obtained shows that on increase in the concentration, the fresh weight increased while minor changes were seen in dry weight.

Discussion

Sodium hypoclorite which is commonly known as chlorate is used as sterilizing agent and effect of NaOCl on germination of various seeds have been studied. Seeds of *A. sellowiana* immersed in sodium hypochlorite at 2% for two minutes reported for 80 % germination [10]. It is also been reported that when NaOCl was used as surface sterilant at lower concentration than 4 per cent resulted in contaminated cultures and when used at higher concentration than 5%, it resulted in the inhibition of germination [9]. In one of the study it was revealed that effective technique for seed surface sterilization in *A. officinalis* was 4% NaOCl for 5 minutes. This techniques significantly affected ($p \leq 0.05$) other characteristic features of seed sterilization [11]. Surface sterilization method for *Ziziphus spina* [Christti] seeds using different concentration of NaOCl showed that sterilization by 4% NaOCl is the best method. In the same experiment it was revealed that seeds treated with 4% NaOCl showed the highest final germination and highest number of sterilized seeds. As use of fungicide and sterilizing solution is an inexpensive means of disease control therefore this method can help in preventing loss of seedling by a variety of fungal pathogens [12]. Above all cited studies showed that treatment of seeds with 4% NaOCl showed highest rate of germination, similarly in our experiment also all four varieties showed maximum germination at 4% NaOCl except for RGC 1002. In present study those varieties of *Cymopsis teragonoloba* was treated, which remained dormant in normal condition of sowing. The previous studies on treatment of seeds of *Dracocephalum moldavica L.* with NaOCl showed increase in percent of germination with increase in concentration [13]. Present study also showed increased germination with increasing concentration of chlorate (NaOCl). It was revealed that RGC 1002 remain unaffected at all tested concentration of NaOCl and showed no germination. Seed germination depends on various factors such as conditions prevailing during seed formation as well as by hereditary factors [14]. In all cases germination percentage were increased with increase in NaOCl concentration. The present study reveals significant effects of NaOCl on three variteis of *Cyamopsis tetragonoloba* out of four studied.

Conclusion

The present study reveals that tested varieties showed stringent seed dormancy and is entirely imposed by the hard seed coat. It is well known that avoidance of germination is ecologically advantageous to the plant growing in harsh climatic conditions, during which seed accumulate and create new population to maintain the species. But it is limiting when quick and high yield of specific variety of seed required. Our results demonstrate that treatment with 4% NaOCL is effective in breaking seed dormancy of seeds of *Cyamopsis tetragonoloba*. During study it was found that RGC 1002 variety remain dormant in control as well treated condition therefore some other method should be explored for breaking dormancy of such seeds.

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Table 1: Effect of NaOCl treatment at different concentration on Guar germination

Concentration of NaOCl (%)	Seed varieties(10 seeds of each)	Germination (%)							
		1 Day	2 Day	3 Day	4 Day	5 Day	6 Day	7 Day	8 Day
0%	RGC 936	0	0	10	10	10	10	10	10
	RGC 1002	0	0	0	0	0	0	0	0
	RGC1003	0	0	20	30	20	20	20	20
	RGC 1017	0	0	0	0	0	0	0	0
0.5%	RGC 936	0	0	10	10	10	10	10	10
	RGC 1002	0	0	0	0	0	0	0	0
	RGC1003	0	20	30	30	40	40	40	40
	RGC 1017	0	0	0	0	0	0	0	0
1%	RGC 936	0	30	30	30	30	30	30	30
	RGC 1002	0	0	0	0	0	00	0	00
	RGC1003	0	40	40	40	50	50	50	50
	RGC 1017	0	0	0	10	10	10	10	10
2%	RGC 936	0	40	40	60	60	60	60	60
	RGC 1002	0	0	0	0	0	0	0	0
	RGC1003	0	50	50	50	50	50	50	50
	RGC 1017	0	0	10	10	10	10	10	10
3%	RGC 936	0	60	60	60	60	60	60	60
	RGC 1002	0	0	0	0	0	0	0	0
	RGC1003	0	80	80	90	90	90	90	90
	RGC 1017	0	0	10	10	20	20	20	20
4%	RGC 936	0	80	80	80	80	80	80	80
	RGC 1002	0	0	0	0	0	0	0	0
	RGC1003	0	100	100	100	100	100	100	100
	RGC 1017	0	0	10	30	30	30	30	30

Table 2: Effect of NaOCl treatment on root & shoot length, fresh weight and dry weight in germinating Guar seedling on 8th day. Values are means ± SEM.

Length & weight	Seeds varieties	Concentration of NaOCl					
		0%	0.5%	1%	2%	3%	4%
Root length	RGC 936	1.45±0.19	1.89±0.11	1.83±0.59	1.89±0.23	2.03±0.58	2.06±0.74
	RGC 1002	--	--	--	--	--	--
	RGC1003	1.66±0.34	1.69±0.28	1.73±0.36	2.08±0.27	3.62±0.24	3.73±0.69
	RGC 1017	--	--	1.47±0.64	2.09±0.29	2.00±0.22	2.08±0.12
Shoot length	RGC 936	2.03±0.26	2.31±0.09	2.44±0.06	2.67±0.73	3.33±0.83	3.42±0.07
	RGC 1002	--	--	--	--		--
	RGC1003	3.79±0.66	4.98±0.57	5.39±0.03	5.77±0.58	5.89±0.55	6.07±0.36
	RGC 1017	--	--	3.64±0.05	3.86±0.24	4.01±0.23	4.09±0.28
Seedling lenth	RGC 936	2.93±0.53	2.32±0.15	4.96±0.04	5.01±0.25	5.16±0.22	5.26±0.76
	RGC 1002	--	--	--	--	--	--
	RGC1003	2.37±0.43	2.73±0.16	2.99±0.06	3.34±0.07	3.77±0.02	3.73±0.69
	RGC 1017	--	--	5.09±0.76	5.03±0.06	6.03±0.13	6.06±0.68
Fresh weight	RGC 936	0.06±0.47	0.8±0.01	0.08±0.03	0.11±0.28	0.12±0.06	0.12±0.02
	RGC 1002	--	--	--	--	--	--
	RGC1003	0.11±0.17	0.13±0.18	0.16±0.06	0.15±0.02	0.15±0.07	0.18±0.06
	RGC 1017	--	--	0.08±0.07	0.10±0.09	0.10±0.03	0.11±0.03
Dry weight	RGC 936	0.01±0.19	0.01±0.03	0.01±0.01	0.03±0.05	0.03±0.01	0.04±0.02
	RGC 1002	--	--	--	--	--	--
	RGC1003	0.01±0.15	0.01±0.08	0.02±0.06	0.02±0.04	0.03±0.09	0.03±0.07
	RGC 1017	--	--	0.05±0.01	0.05±0.06	0.05±0.02	0.06±0.05

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