The effects of sodium azide on seed germination and seedling growth of chili pepper (Capsicum annum L. cv. Landung)

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IOP Conference Series: Earth and Environmental Science

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To cite this article: Yafizham and B Herwibawa 2018 *IOP Conf. Ser.: Earth Environ. Sci.* **102** 012052

The effects of sodium azide on seed germination and seedling growth of chili pepper (*Capsicum annum* L. cv. Landung)

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Abstract. This study was aimed to determine the effects of sodium azide on the performance of chili pepper. Dry seeds from pure lines of chili pepper cv. Landung were used in this experiment. Each set containing 50 seeds was placed in nylon fishner bags, washed in flowing water and soaked in distilled water for four hours. After soaking, four sets of seeds were dabbed dry with tissue paper and were treated for two hours at 30 ^{0}C in appropriate solution of 0, 0.10, 0.20, 0.40, 0.80, 1.60 mM sodium azide with phosphate buffer at pH 3. After treatment, the seeds were germinated in plastic boxes containing sterilized sand and kept under laboratory condition by supplied with water everyday. The seeds for M_1 germination percentage, seedling height, plant height and number of leaves were recorded 30 days and 60 days after treatment, respectively. Seeds which radicle emerged were considered germinated, the seedling and plant height were measured from the tip of primary root to the base of the first leaf pair, and the number of leaves were counted for only fully expanded leaves. A completely randomized block design in four replicates was used throughout the experiment. Data obtained were analysed for range, mean, standard of deviation, and percent of control using Microsoft Office Excel 2007 software. It was concluded that different doses of sodium azide influenced the performances of chili pepper cv. Landung. Very low doses of sodium azide (0-1.60 mM) might be used to study the improvement of chili pepper diversity.

Keywords : sodium azide, chili pepper, germination

1. Introduction

The mutagenicity of sodium azide has been demonstrated in several plants, viz. maize [1], rice [2], wheat [3], chickpea [4], fenugreek [5], sunflower [6], rapeseed [7], tomato [8], etc. Inconsistent effects of sodium azide have been obtained in chili pepper, and it is well known that there are many factors such as varieties, ages and environmental factors [9,10,11]. There are varying opinions as to mutagenic properties of sodium azide, however, it has been reported that hydrogen ion concentration of its treatment solution as a key to its high mutagenic potency [12]. Mutagenic efficiency of sodium azide is also possiby due to the low physiologic damage azide induced in treated plants [13].

The mutagenicity of the sodium azide to an indirect action, through its inhibition of catalase and peroxidase were caused by the accumulation of hydrogen peroxidase [14]. Many studies demonstrated the sodium azide must be metabolized by plant cells to the mutagenic agent, identified as an amino acid analogue L-azidoalanine (N_3-CH_2-$CH(NH)_2$-COOH), and the production of this metabolite was

IOP Conf. Series: Earth and Environmental Science **102** (2018) 012052 doi:10.1088/1755-1315/102/1/012052

found on the O-acetylserinesulfhydrylase, in which the enzyme catalyses the condensation of azide or sulfide with O-acetylserine to produce azidoalanine or L-cysteine respectively [15]. The pH of the solution influences the mutagenic efficiency of sodium azide, and it has been shown that azide is most effective in inducing mutations at pH 3 [13].

The increasing work on the induction of mutations to increase genetic diversity using mutagen reflects the growing interest in mutation breeding [16]. It is also indicative of the mutagenic efficiency of sodium azide. However, there is no work done in the mutagenicity of sodium azide on chili pepper cv. Landung. Its use in mutation breeding may help improve the existing varieties of the capsicums. Mutant selection would be done since germination phase [17]. The characters of germination, as measured in the laboratory, is able to reflect the ability of plants to live under field conditions [18]. This study was aimed to determine the effects of sodium azide on the performance of chili pepper. The M_1 germination percentage, seedling height, plant height and number of leaves were recorded 30 days and 60 days after treatment, respectively. This study is expected to provide information that can be used in chili pepper breeding programs.

2. Materials and Methods

Dry seeds from pure lines of chili pepper cv. Landung were used in this experiment. A modified methods by [11] were employed. Each set containing 50 seeds was placed in nylon fishner bags, whased in flowing water and soaked in distilled water for four hours. After soaking in water, four sets of seeds were dabbed dry with tissue paper and were treated for two hours at 30 ^{0}C in appropriate solution of 0, 0.10, 0.20, 0.40, 0.80, and 1.60 mM sodium azide with phosphate buffer at pH 3. After treatment, the seeds were washed for 2 hours in running water and then germinated in sand sterilized for one hour at 16 p.s.i in plastic boxes and kept under laboratory condition by supplied with water everyday.

The seeds were scored for M_1 germination percentage and seedling height 30 days later, and plant height and number of leaves 60 days after treatment. Seeds which radicle emerged were considered germinated, the seedling and plant height were measured from the tip of the primary root to the base of the first leaf pair, and the number of leaves were counted for only fully expanded leaves. The entire experiment was arranged in a completely randomized block design in four replicates. Data obtained were analyzed for range, mean, standar of deviation, and percent of control using Microsoft Office Excel 2007 software.

3. Results and Discussions

Sodium azide decreased germination percentage of chili pepper (Table 1). It was found that the germination percentage ranged from 28 to 100 % and decreased 1.01 to 2.96 times. Similar experiments have been carried out in chili pepper by [10] and [11]. The germination percentage of chili pepper cv. pusa jwala and cv. hyderabad in different doses were detected from 40 to 90 %. It was explained the sensitivity to sodium azide was doses dependent, and evident that sodium azide produced physiological effects. The effect of sodium azide was also noticeable in growth inhibition as a very common effect in chili pepper [9].

Table 1. Germination percentage of seeds of chili pepper cv. Landung after sodium azide treatments

Sodium Azide (mM)	Germination Percentage (%)		Percent of control
	Range	Mean/SE	
0	99 – 100	99.67 ± 0.58	100.00
0.10	93 – 94	93.33 ± 0.58	93.65
0.20	90 – 93	91.33 ± 1.53	91.64
0.40	83 – 92	87.00 ± 4.58	87.29
0.80	67 – 80	73.67 ± 6.51	73.91
1.60	28 – 42	33.67 ± 7.37	33.78

Table 2. Seedling height of chili pepper cv. Landung after sodium azide treatments

Sodium Azide (mM)	Seeding Height (cm)		Percent of control
	Range	Mean/SE	
0	3.27 – 3.90	3.59 ± 0.32	100.00
0.10	2.30 – 3.70	3.13 ± 0.74	87.28
0.20	2.10 – 3.50	2.84 ± 0.70	79.20
0.40	2.00 – 3.20	2.67 ± 0.61	74.28
0.80	1.80 – 2.90	2.39 ± 0.55	66.57
1.60	0.59 – 1.84	1.23 ± 0.63	34.26

There is little information on the mechanism of azide mutagenesis in chili pepper. However, it has been reported that sodium azide causes the reduction of plant height of rice [19] and wheat [3]. The reports support the findings of the experiment, which is sodium azide reduced seedling height (Table 2) and plant height (Table 3). Different results shown in sunflower [20], grapevine [21], and lentil [22] where the plant height reduction is not consistent with increase of sodium azide doses. It is common knowledge that mutations whether they are point or gross chromosomal aberrations involve alterations in the DNA molecule. Alterations in the DNA structure and constituent nucleotides brought about by mutagens may change the genetic information, and possible to give different expressions.

Table 3. Plant height after 60 days of chili pepper cv. Landung after sodium azide treatments

Sodium Azide (mM)	Plant Height (cm)		Percent of control
	Range	Mean/SE	
0	14.30 – 15.70	14.93 ± 0.71	100.00
0.10	13.40 – 14.70	14.02 ± 0.65	93.91
0.20	12.80 – 13.40	13.11 ± 0.30	87.79
0.40	12.30 – 13.30	12.77 ± 0.50	85.49
0.80	11.60 – 12.40	11.98 ± 0.40	80.20
1.60	4.15 – 5.81	5.16 ± 0.89	34.58

Table 4. Number of leaves after 60 days of chili pepper cv. Landung after sodium azide treatments

Sodium Azide (mM)	Number of Leaves		Percent of control
	Range	Mean/SE	
0	9.20 – 13.10	11.14 ± 1.95	100.00
0.10	7.80 – 10.20	8.99 ± 1.20	80.68
0.20	8.70 – 10.00	9.39 ± 0.65	84.27
0.40	8.20 – 10.30	9.30 ± 1.05	83.46
0.80	8.00 – 11.60	9.27 ± 2.21	83.16
1.60	3.75 – 5.43	4.66 ± 0.85	41.82

The highest number of leaves (11.14) was observed for control (0 mM) and the lowest (4.66) with 1.60 mM (Table 4). The reduction of number of leaves was similar to research [23] and [24] of black rice and tomato, repectively, where the number of leaves decreased as the higher of sodium azide doses. However, these results were different with those obtained by others, the reduction of number of leaves was not consistent with increased in sodium azide doses of rice [25] and groundnut [26]. The inhibitory effects of sodium azide on the different biological parameters of chili pepper may probably be explained by the inhibition of mitosis, disruption of the enzymatic process or direct changes on the genes involved [27]. There are different mechanism among the given mutagen doses, so it caused changes of each individual [28]. It was evident from the results obtained that there were differences response to sodium azide were doses and individual chili pepper plant dependent. These may be attributed to difference in the uptake or metabolism of the mutagen, cellular repair mechanisms or differences in the individual metabolic activities.

4. Conclusion

It was concluded that different doses of sodium azide influenced the performances of chili pepper cv. Landung. Very low doses of sodium azide (0-1.60 mM) might be used to study the improvement of chili pepper diversity.

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