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Research Article

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Mutagenic Effect of Sodium Azide (NaN_3) on Seed Germination and Chlorophyll Content of *Spinach oleracea*

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ABSTRACT

Chemical mutagenesis is an efficient tool used, to induce mutation for improving various characteristics in plants. In this study sodium azide with three concentrations (0.01- 0.03%) were used to analyse their effect on seed germination and chlorophyll content. It was noted that increase in the dose of sodium azide, germination percentage was decreased but total chlorophyll content was increased. The maximum chlorophyll content was observed under 0.03%. So, the effect of chemical mutagenesis is much more beneficial and it is effective for chlorophyll content. Hence this mutagen can be used for improving this character.

Keywords: *Spinach, Mutagenesis, Sodium Azide, Chlorophyll content.*

INTRODUCTION

Mutation breeding has been widely used for the improvement of plant characters in various crops. It is a powerful and important tool for plant breeding especially for autogamous crops having narrow genetic base (Micke, 1988). The use of mutagens in crop improvement helps to understand the mechanism of mutation induction and to quantify the frequency as well as the pattern of changes (Mensah et al., 2007). Induced mutations have been successfully utilized to improve yield and components of various crops like Oryza sativa (Rao & Siddiq, 1977), H.vulgare (Ramesh et al., 2001), Vigna unguiculata (Mensah & Akomeah, 1992, Mshembula et al., 2012), Cajanus cajan (Srivastava & Singh, 1996), Vigna mungo (Kundu & Singh, 1981, and Singh & Singh, 2001), Lens culinaris (Rajput et al., 2001 & Khan et al., 2006). It is known that various chemicals have positive or negative effects on living organisms. Chemical mutagen generally produce induced mutations which lead to base pair substitution especially GC→AT (guanine:cytosine to adenine:thymine) resulting in amino acid changes, which change the function of proteins but do not abolish their fun ctions as deletions or frame shift mutations mostly (Al Qurainy, 2009 & Khan et al., 2009).

Sodium azide (NaN_3) is a colorless, odorless and crystalline solid (Nilan et al., 1977). It is relatively safe to handle, inexpensive, non carcinogenic and most efficient mutagen in crop plants.

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It is a common bactericide, pesticide and industrial nitrogen gas generator if known to be highly mutagenic in several organisms, including plants and animals (Rines, 1985 and Grant & Salomone, 1994). In order to understand that sodium azide is mutagenic mechanism used for the improvement economic characters to many studies in rice, wheat, barley and sorghum (Maluszynski et al., 2009).

Leafy vegetables have become an important part of the diet because of their nutritive value and low calorie content. Plant leaves are one of the most important sources of medicines. Spinach (Spinacia oleracea) is a leafy green flowering plant native to central and Western Asia. It belongs to family Amaranthaceae. Leaves are common edible vegetable consumed either fresh or after storage. Spinach is a super food with tons of nutrients in low calorie package. Spinach can be added as an ingredient to many dishes and either cooked or served raw. Raw spinach has 91% water, 4% carbohydrates, 3% protein and contains negligible fat. It is a rich source of vitamin A, C, K and E, Calcium, Potassium, Magnesium, Manganese, Iron, folate and dietary fiber. Apart from having nutritional value, it has been also credited with various biological activities like virus inhibitor (Adam et al., 2008) anthelmentic (Patile et al., 2009) and antioxidant (Verma et al., 2003) hepatoprotective (Gupta & Singh, 2006) and reducing risk of breast cancer (Longnecker et al., 1997).

Chlorophyll mutation are considered as the most important parameter for evaluating the efficiency of different mutagens in inducing the genetic variability for crop improvement and are also used as genetic markers in basic and applied research (Saad-Allah et al., 2014).

This study was aimed to determine the effect of sodium azide on seed germination and to the chlorophyll content of spinach. This study is expected to provide information that can be used in spinach breeding programs.

MATERIALS AND METHODS

Seeds of spinach obtained from local market of Jaipur. The seeds were surface sterilized using 0.01% (w/v) mercuric chloride for 8 minutes, with continuous stirring, rinsed thoroughly several times in distilled water. Then three groups of them were soaked in three concentrations of sodium azide (0.01%, 0.02% and 0.03%) for 4 hours and control was maintained by soaking the seeds in distilled water. The treated seeds were washed in running water to remove excess chemicals from the seeds and sown in pots. The pots were left until maximum seed germination was attained and the germination percentage was calculated.

Chlorophyll determination

The chlorophyll content of the control and treated leaves was carried out by following the methods of Arnon (1949). Leaves were harvested from plant of 30 days of age.

RESULT AND DISCUSSION

Percentage germination in the control experiment was 100%. There were reduction in the germination and survival percentages with increasing concentrations (Table1) of sodium azide treatment. It was directly proportional to the dosage. Reductions in seed germination may be due to the delaying or inhibition of physiological and biological processes necessary for seed germination which include enzyme activities and to the inhibition of mitotic process (Mensah et al., 2006). The effect of sodium azide on seed germination could be due to azide anions. These anions are strong inhibitors of cytochrome oxidase which later on inhibits oxidative phosphorylation. It is a potent inhibitor of the proton and alters the mitochondrial membrane potential. These effects together may hamper ATP biosynthesis resulting in decreased availability of ATP which may slow the germination rate and reduce the germination percentage (Kleinhofs and Sander 1975; Zhang 2000 and Cheng and Gao 1988). Effect of sodium azide on seed germination and plant survival was reported in Stevia rebaudiana (Pande & Khetmalas 2012), Nigella, Plantago and Trigonella by Prabha et al., 2011. Morphological variations were also reported by Pearson et al., (1974 and 1975) in

Barley, Fay (1975) in wild oats, Srivastava et al. (2011) in wheat and Adebola (2013) in tomato.

The total chlorophyll, chlorophyll a and chlorophyll b content was affected at all studied concentrations. Plants developed with the seed, pre-soaked in the sodium azide solution initially caused a decrease in chlorophyll 'a', chlorophyll 'b' and total chlorophyll content in 0.01% and then show an increase in all content at 0.02% and 0.03% (Table1, Fig.1). It also delay the senescence of leaves. NaN_3 was shown to be a potent mutagen in Barley and induced chlorophyll deficiency as well as a wide range of morphological and physiological mutants (Kleinhofs et al., 1978). Reduction in chlorophyll content may due to the disturbances at some stage in chlorophyll apparatus (Mensah et al., 2006) or to chlorophyll mutations (Gaibriyal et al., 2009). This reduction in chlorophyll content may also b from inactivation of enzymes responsible for chlorophyll biosynthesis (Rahman et al., 2005).

The high content of chlorophyll was found at higher concentrations (0.02% and 0.03%) as compared to the control plant. This variability in chlorophyll content may be the mutation at various loci of genome at various concentrations of sodium azide. Owais and Kleinhofs (1988) stated that the NaN_3 caused mutation in arbitrated through the formation of an organic metabolite which enters the nucleus, interacts with DNA and generates point mutations in the genome. Utilization of NaN_3 in generating genetic variability in plant breeding has been reported in Barley (Kleinhofs & Sander, 1975), in Groundnut (Mensah & Obadoni, 2007) and other crops (Avila & Murty 1983, Rautaray et al., 1995). The main advantage of mutation breeding is the possibility of improving one or two characters without changing the rest of the genotype. The results of present study suggest that particular dose of NaN_3 can be used to create genetic variability in spinach, which could be the basis for quality improvement in this species.

Table 1: Effects of sodium azide (NaN_3) on seed germination and chlorophyll content

	Total Chlorophyll	Chlorophyll 'a'	Chlorophyll 'b'	Germination percentage (%)
Control	0.245	0.159	0.086	100
0.01%	0.222	0.14	0.082	81
0.02%	0.412	0.177	0.235	72
0.03%	0.417	0.18	0.237	60

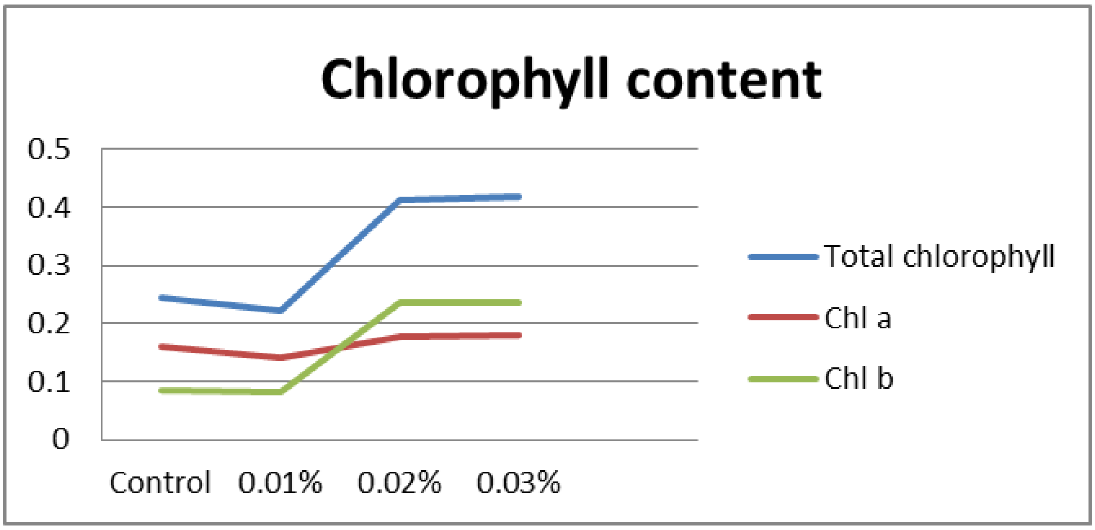

Fig. 1: Effect of sodium azide on chlorophyll content

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