International Journal of Theoretical & Applied Sciences, 10(1): 169-173(2018)

ISSN No. (Print): 0975-1718
ISSN No. (Online): 2249-3247

Comparative Mutagenic Effectiveness and Efficiency of Sodium Azide and Gamma Radiation in Onion (*Allium cepa* L.)

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(Received 03 January 2018, Accepted 18 March, 2018)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The present investigation deals with the studies on the comparative mutagenic effectiveness and efficiency of chemical mutagen sodium azide (0.1-0.7% for 4hrs) and physical mutagen gamma radiation (2-12kR) alone and in combination in onion (*Allium cepa* L.) var. N-2-4-1. To calculate mutagenic effectiveness and efficiency, the data obtained on biological damage of M_1 in terms of lethality and sterility, chlorophyll mutations induced in M_2 generation were used. The results obtained revealed that, sodium azide alone treatment was most effective. The results on efficiency, on the basis of lethality, indicates that, gamma radiation and on the basis of pollen sterility, combined treatments showed high efficiency than the alone treatments of physical and chemical mutagens.

Key words: Onion (*Allium cepa* L.), Mutagenic effectiveness and efficiency, Sodium azide, Gamma radiation.

I. INTRODUCTION

Vegetables are the important component of the daily diet. Onion is an economically important vegetable and source of foreign currency for the country. It is cultivated for the bulbs, which are used for culinary, vegetable and medicinal properties since prehistoric time.

Mutation breeding has contributed significantly to the global biodiversity, agriculture and human welfare by producing more than 3000 mutant varieties with enhanced production and productivity in a large number of crop species (175). Some of the leading countries e.g., China, India, Japan, USA, Vietnam, France, Pakistan, Bangladesh, Bulgaria, Canada, Italy, Brazil, Spain, U.K., Poland and Portugal which have made spectacular accomplishments in evolving several superior mutants and released large number of mutant varieties in some of the most important agricultural crops through the use of induced mutagenesis [1-4]. Several of these mutant varieties are being cultivated by farmers in millions of acres in large number of countries in all the continents all over the world. The attempts have been made by various workers to understand the role of induced mutations in onion through chemical and physical mutagens [5-15]. They reported that the morphological, physiological characters were altered, seed yield, bulb yield and TSS was also improved. Four mutant varieties of onion namely Compas, Brunette, KIK-11 and KIK-13 were released in Netherlands and Russia for cultivation [5]. The improved characters were earlyness, stiffness and bulb yield.

In induced mutations, the difference in mode of action of mutagen [12], efficiency of repair process [17] and factors affecting the expression of concerned mutations with varying frequency and spectrum [18] are also equally important. Efficiency and effectiveness are the important parameters to evaluate the usefulness of the mutagen. Effectiveness of the mutagen is the number of mutations produced per unit of the mutagen conc./dose while efficiency is the ratio of specific desirable mutagenic changes to undesirable effects such as lethality, sterility, chromosomal aberrations etc. in M_1 generation. The present investigation was undertaken to study effectiveness and efficiency of sodium azide and gamma radiation, alone and in combination treatments in onion.

II. MATERIALS AND METHODS

For present investigation, the seeds of onion var. N-2-4-1 obtained from National Research center for Onion and Garlic, Rajgurunagar, Pune (MS) were used.

A. Mutagenic Treatments

Gamma radiations: Truely breeding, completely dry, clean and uniform seeds of onion var. N-2-4-1 were exposed to different doses of gamma radiations in the Department of Nuclear chemistry, University of Pune, Pune-411007. The source of gamma radiations was ^{60}Co and the doses were as –2, 4, 6, 8, 10 and 12 kR.

Sodium azide: The seeds of onion soaked in distilled water for 12hrs and then treated with the different concentrations i.e. 0.1%, 0.3%, 0.5% and 0.7% of sodium azide for four hours.

Combination treatments: For combination treatments, gamma radiations and sodium azide, in different doses and concentrations were used. The dry seeds were first exposed to different doses of gamma radiations and then soaked in distilled water for 12hr. The soaked seeds were blotted to dry and then treated with different concentrations of sodium azide.

The seeds treated with chemical mutagens were washed in running tap water for 1hr. to leach out the residual chemicals. Dry seeds were used as Control.

B. Raising of M_1 generation

The life cycle of the onion is completing in two phases 1) Bulb production and 2) seed production. For bulb production seeds were sown and for seed production bulbs were used. The details are as follows.

Sowing of the seeds for bulbs production. Treated and control seeds were sown on the same day in well prepared nursery beds of experimental field of National Research center for Onion and Garlic, Rajgurunagar, Pune (MS). The seedlings were transplanted after 45-50 DAS. The treated and control populations were maintained under uniform climatic and agronomic conditions till the harvesting of bulbs.

Plantation of bulbs for seed production. After harvesting and curing, bulbs were randomly selected from each treatment and control and used for seed production. Each plant was bagged to ovoid cross population. Umbels of the stalks were shaken every day manually for effective pollination. Seeds were harvested separately from each plant and stored in paper bags. These were used for sowing for next generation.

In M_1 generation the parameters- lethality, pollen sterility were studied. This data was used to calculate mutagenic efficiency. The M_2 population was screened keenly for chlorophyll mutations. The data on frequency of chlorophyll mutations was obtained and used to calculate mutagenic effectiveness.

C. Estimation of Mutagenic effectiveness and Efficiency

Mutagenic effectiveness is a measure of the frequency of the mutations induced by a unit dose of mutagens (kR or time x concentration/dose) while mutagenic efficiency gives an idea of the damage such as lethality, pollen sterility etc. in M_1 generation.

Mutagenic effectiveness and efficiency of different mutagens were calculated according to formulae [19].

For chemical and physical mutagen:

Mutagenic effectiveness - Mf/tc or Mf/kR

Mutagenic efficiency- Mf/L or Mf/S

Where,

Mf = % of chlorophyll mutations in M_2 generation

t = Period of treatment

c = Concentration of Chemical mutagen in terms of %

kR = Kilo rad of physical mutagen

L = % of lethality in M_1 generation

S = % of pollen sterility

III. RESULTS AND DISCUSSION

A. Mutagenic effectiveness

Effectiveness is number of mutations produced per unit dose/conc. In M_1 generation the values for the effectiveness of sodium azide were ranging from 2.05 to 7.02. Average effectiveness of sodium azide was 3.73. The highest value was noted in 0.1% SA, while the lowest value recorded in 0.7 % SA.

The results of gamma radiation indicated that, the range of effectiveness was 2.18 to 2.77 while average effectiveness of gamma radiation was 2.44. The highest effectiveness was recorded in 8-kR dose treatment, while the lowest effectiveness at the dose 4kR.

The data recorded regarding the effectiveness of the combination treatments of SA and gamma radiations revealed that, the highest effectiveness was found at 6kR + 0.5% SA while the lowest at 4kR+0.1%. The range was 2.0–4.08 while average effectiveness was 3.08.

From Table 1 -The trend of mutagenic effectiveness was –

SA (3.73) **>** combination treatments (3.08) **>** gamma radiations (2.44).

Overall results obtained indicated that, sodium azide was most effective than the gamma radiation alone and in combined treatments. Similar results were reported by other worker in rice, winged bean, Horse gram and cluster bean [20-24] and concluded that Sodium azide was most effective than the other chemical as well as physical mutagen. Chemical mutagens are also most effective than the physical mutagens [25-26].

B. Mutagenic efficiency

Efficient mutagenesis involves the aim to produce desirable changes free from association with undesirable effects [19]. Thus, efficiency of the mutagen is nothing but the ratio of specific changes to undesirable effects like plant damage, sterility, lethality, survival etc. Some parameters which can be used as 'reasonable quantitative measures' of the relative efficiency of different treatment like the number of mutations (for which often number of chlorophyll mutations are taken) per 100 M_1 spike (or 100-M_2 seedlings), divided by the percentage of injury, lethality or sterility [19].

High efficiency of mutagen obtained only when the mutagenic effects greatly surpasses the damage in cells [19]. The efficiency is parameter which gives highest mutation rate due to mutagenicity [27].

Table 1: The effectiveness and efficiency of sodium azide and gamma radiation alone and in combination in onion (*Allium cepa* L) var. N-2-4-1.

Treatments	Frequency of Chlorophyll mutations	Mutagenic Effectiveness	% Lethality	Mutagenic Efficiency	% Sterility	Mutagenic Efficiency
Sodium azide (%)						
0.1	2.81	7.02	18.18	0.15	12.57	0.22
0.3	3.73	3.10	20.66	0.18	9.14	0.40
0.5	5.55	2.77	26.00	0.21	69.27	0.080
0.7	5.75	2.05	31.24	0.18	10.39	0.55
	Average	3.73	4.80	0.18	25.34	0.31
Gamma Radiations (kR)						
2	4.66	2.33	23.80	0.19	7.10	0.65
4	8.74	2.18	28.80	0.30	7.19	1.21
6	16.44	2.74	30.82	0.53	8.47	1.94
8	22.19	2.77	32.63	0.68	9.82	2.25
10	24.19	2.41	34.80	0.69	14.40	1.67
12	27.22	2.26	44.83	0.60	18.76	1.45
	Average	2.44	32.61	0.49	10.95	1.52
Gamma radiations (kR) + Sodium azide (%)						
2+0.1	09.53	3.97	33.86	0.28	3.54	2.69
2+0.3	12.26	3.83	53.60	0.22	4.34	2.82
2+0.5	15.67	3.91	62.72	0.24	7.41	2.11
4+0.1	08.83	2.0	31.33	0.28	9.81	0.90
4+0.3	14.18	2.72	41.30	0.34	13.33	1.06
4+0.5	16.56	2.76	43.30	0.38	15.17	1.09
6+0.1	16.82	2.62	47.61	0.35	8.05	2.08
6+0.3	23.40	3.25	51.87	0.45	11.31	2.06
6+0.5	32.71	4.08	37.78	0.85	16.63	1.96
8+0.1	18.43	2.19	62.62	0.29	10.27	1.79
8+0.3	25.15	2.73	63.87	0.39	17.26	1.45
8+0.5	29.78	2.97	73.83	0.40	26.00	1.14
	Average	3.08	50.30	0.37	11.91	1.76

Table 2: Comparison of effectiveness and efficiency of mutagen in onion (*Allium cepa* L) var. N-2-4-1.

Mutagen	Effectiveness	Efficiency on the basis of	
		Lethality	Sterility
Sodium azide	3.73	0.18	0.31
Gamma radiation	2.44	0.49	1.52
Sodium azide + Gamma Radiation (Combination treatments)	3.08	0.37	1.76

The trend of efficiency was as follows-
On the basis of lethality (Table 2) –
Gamma radiations (0.49) > Combined treatments (0.37) > SA (0.18)
On the basis of sterility (Table 2) –
Combined treatments (1.76) > Gamma radiation (1.52) > SA (0.36)

Efficiency with reference to Lethality. In sodium azide treatment, the efficiency with reference to lethality showed increased trend (0.15–0.21) up to 0.5% SA treatment while at higher concentration i.e. (0.7%), it was again decreased (0.18). The average efficiency was 0.18. For gamma radiations, the range was 0.19 to 0.69 while average was 0.49.

The trend was similar to that of sodium azide. As compared to sodium azide, gamma radiations showed higher efficiency. The results of combined treatments of gamma radiation and sodium azide indicated that, the average efficiency was 0.37 and it ranged from 0.22 to 0.85. But there was no definite pattern observed regarding lower dose (2kR) of gamma doses combined with different concentrations of sodium azide. Concentrations of the sodium azide showed gradual increase with the increasing concentrations of sodium azide. Highest value of the efficiency was observed in 6kR + 0.5% SA treatment. As compared to 6kR, the higher dose *i.e.* 8-kR combined with different concentrations of sodium azide, the efficiency was decreased (0.40).

Efficiency with reference to Sterility. In M_2 generation, the average efficiency was 0.36 and it ranged from 0.080 to 0.55 in sodium azide treatments. Except the 0.5% SA, the efficiency was showing increased trend. Very low efficiency was recorded at 0.5% sodium azide treatment *i.e*. 0.080. For gamma radiation dose treatment the highest efficiency was recorded at 8kR (2.25). The range was 0.65-2.25 while average was 1.52. There was gradual rise in efficiency till 8kR and it was again declined at higher doses (10kR and 12kR) recording 1.67 and 1.45 respectively. The average efficiency of combined treatments was 1.76. Among all the combined treatment, 2kR + 0.3% SA, 2kR + 0.1% SA, 2kR + 0.5% SA and 6kR + 0.1% SA were showing highest values such as 2.82, 2.69, 2.11 and 2.08 respectively.

In present investigation gamma radiation found to be more efficient on the basis of lethality. Similar results were reported by other workers in Soybean and Cluster bean [28-29]. On the basis of Sterility, combined treatment of gamma radiation and sodium azide, it was highest as compare to gamma radiation and sodium azide alone treatment. The results are conformity with the findings of other worker [30].

Thus, overall results, it can be conclude that sodium azide was most effective mutagen than gamma radiation alone and in combination treatments. The mutagenic efficiency varied with different biological parameters i.e. lethality and pollen sterility were studied. Regarding this, gamma radiation and combination treatments were most efficient treatments based on lethality and pollen sterility respectively.

ACKNOWLEDGEMENT

I extend my grateful thanks to Dr. K. N. Dhumal (Ex. Professor in Botany, University of Pune, Pune), for his valuable guidance and constant encouragement. I am also thankful to Dr. K. E. Lawande, (Ex. Director, NRC, Onion and Garlic, Rajgurunagar, Pune) for providing the seed material, field facility and constant support.

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