Research Article

RESPONSE OF IRRIGATION AND PLANT BIO-REGULATORS SPRAY ON SEED YIELD AND QUALITY OF EGYPTIAN CLOVER (*Trifolium alexandrinum* L.)

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ABSTRACT
Lower seed yield and quality is one of the major issues of the Egyptian clover cultivation in Nepal. In order to develop technologies to increase the seed yield, and to improve the seed quality, an experiment was done in the Regional Agricultural Research Station, Khajura, Banke, Nepal during November, 2017 to May, 2018, by using Randomized Complete Block Design with four replications. Treatments composed of two factors combination of irrigation (zero irrigation, and two times irrigations after cutting for fodder); and spraying of plant bio-regulators (no spray, and two times foliar spray of plant bio-regulators at the pre-flowering stage). The plot size was 12 m^2. The parameters measured included seed yield, biological yield of whole plant above ground level, seed attributing characters, and seed quality. The irrigation and application of plant bio-regulators alone, significantly ($p<0.05$) increased the seed yield, biological yield, harvest index, and major attributing characters of the seed. Thus the results of this experiment revealed that providing two irrigations to the Egyptian clover in 15 days interval after taking one cutting for fodder in 60 days after sowing, and two times foliar spray of plant bio-regulators in seven days interval at pre-flowering stage could increase its seed yield and overall seed quality aspects.

Key words: Seed attributing characters, germination, test weight, seedling vigor

INTRODUCTION

Egyptian clover (*Trifolium alexandrinum* L.), popularly known as the berseem or berseem clover, is an important winter fodder legume in Nepal. It has been one of the vital constituents of the winter feeding strategies to the ruminants of Terai, and sometimes middle hills for more than four decades (Sharma, 2015). Due to its good fodder quality in terms of nutrient, palatability etc. and high biomass yielding capacity in its five to six cuts over a long growing season from November to May, it is referred as the king of the fodders (Tiwari & Yadav, 2014). It is popularly growing for fodder as relay crop with paddy, mixed crop with oat and as main crop and monoculture in cultivated lands (Pande, 1995).

Egyptian clover is no longer a new crop for farmers of Nepal for seed production also. With the initiation of the activities on the development of fodder and pasture since 1970, many potential fodder species and cultivars including Egyptian clover are being introduced and cultivated. It became the popular among farmers due to its high nutritional value, multi-cut regime, and ease of cultivation of many introduced fodders. The Egyptian clover seed was used to be produced in various 11 districts of Nepal few decades ago when more than 7 t of seed were produced each year (Pande, 1995). Although, the crop is being more popular over the years for fodder production in Terai regions of Nepal presently covering more than six thousands hectares (NAFLQML, 2019), the farmers are more interested to grow for its green biomass rather than for seeds. As a result, the seed production is decreased in a greater extent in the recent years which are not being able to fulfill the demand of the Egyptian clover seed in Nepal. The context is consequential to import of the large quantity of the Egyptian clover seed from India, Australia, New Zealand and other countries every year.

On the other hand, there are some pocket areas with good potentiality of Egyptian clover seed production inside the country. The crop is considered suitable in the southern belt of Dhanusha, Mahottari, Sarlahi and in Dang, Banke and Bardiya for seed production from an agro-climatic point of view as a main crop or as a relay crop with paddy. It is estimated that approximately 0.2 million ha of paddy land could be used to grow Egyptian clover seed in these districts, which could produce more than 40,000 t of seed (Pande, 1995).

This crop is one of the high yielder of seeds compared to other species of fodders. The seed multiplication ratio is about 25-30 times. Under Nepalese conditions, with specific dose of fertilizers and irrigation regimes, seed production ranges from 240-783 kg ha^{-1}. In the farmers' situation, it was observed that the normal seed production is about 100-120 kg ha^{-1} due to some insufficient and inappropriate management practices (Pande, 2014). It indicates the tremendous potentiality of the Egyptian clover seed production in the country in order to substitute current import.

Good quality of seed ensures higher yield of seed and biomass. But, quality of the seed is another problem of the lower seed and biomass production of Egyptian clover in Nepal. Most seeds are of poor quality, either produced at the government farm or at the farmer's level. There is immense need of increasing the seed productivity and quality (Pande, 2014).

Although, there is a wide scope and tremendous potential to grow Egyptian clover seed in Nepal, there is huge gap between seed demand and supply from the seed produced inside the country (Sharma, 2015). Some achievements were made in increasing the seed yield of Egyptian clover by governmental and non-governmental sectors, but numbers of issues for research are still remain. So, this experiment was done to analyze the effect of irrigation management, and spray of plant bio-regulators in terms of increasing the yield and the quality of Egyptian clover seed in Mid-western Terai regions of Nepal.

MATERIAL AND METHODS

The experiment was carried out from November 2017 to May 2018 in the experimental site of Regional Agricultural Research Station, Khajura, Banke at 28.11336 N, 81.59199 E and at the elevation of 155 masl. The soil was sandy loam with 6.8 pH. The experiment was done using Randomized Complete Block Design (RCBD) with factorial arrangements of 2×2 factors, and three replications. Zero irrigation, and 2-irrigations (at the rate of 666.67 kiloliters of water ha^{-1}) at 15 days interval after taking the one-cut at 60 days of plant age were two levels of the irrigation factors. Likewise, no spray of plant bio-regulators, and 2-foliar sprays of plant bio-regulators (P_2O_5 @ 2 kg ha^{-1}) + KNO_3 @ 4 kg ha^{-1}) at one week interval on pre-flowering stage were two levels of the bio-regulators spray. The plot size of 12 m^2 was taken as an experimental unit.

In executing the experiment, plant establishment procedures and agronomic practices were done as suggested by Bijaya et al. (2017). Continuous line sowing was adopted with 40 cm row to row spacing. The seed was sown at 3 to 4 cm depth with the seed rate of 20 kg seed ha^{-1}. A total of 20 kg N, 60 kg P_2O_5 and 40 kg K_2O were added in the soil for nutrient management at the time of land preparation. Weed management was done manually in 15 days and 30 days after sowing and as per the requirement. The seeds were inoculated with *Rhizobium trifolii* prior to sowing. One-cutting was taken for green fodders at 60 days after sowing from each plot and plants were left for seed production.

The observations for seed weight, plant biomass above the ground level, number of inflorescence m^{-2}, number of florets inflorescence^{-1}, number of florets plant^{-1}, number of viable nodules plant^{-1}, days to flower initiation, days to 50% flowering, days to maturity were taken from the field. Number of viable nodules were calculated by counting the Red (*R-type*) nodules which was considered as Bacteroid-filled with haemoglobin-pigmented bacterial tissues. Likely, seed germination %, test weight (1000 seeds) and vigor index were determined in the laboratory of Seed Science and Technology Division under Nepal Agricultural Research Council, Khumaltar, Lalitpur. The test weight of hundred seeds in eight replications in first test and eight replications in second test from every plot samples were counted, weighed and average thousand seeds weight were calculated and expressed in gram.
Seedling vigor indices were calculated by using formula suggested by Abdul-Baki & Anderson (1973).
Seedling vigor index = Standard germination (%) x Seedling dry weight (mg).
The data was analysed by using R-Packages (2013) software.

RESULTS

Seed yield and yield attributing characters

The seed yield, biological yield and the harvest index for the irrigation and plant bio-regulators spray is presented in Table (1). The seed yield of Egyptian clover with 2-irrigations was higher ($p<0.01$) in comparison to the seed yield of the treatments of zero irrigation. Similar case was obtained in the case of biological yield also. The treatments with 2-irrigations had yielded better ($p<0.01$) biological yield than the treatments with zero irrigation. Likely, better harvest index was obtained for the treatments with 2-irrigations in comparison to zero irrigation treatments.

Table 1. Effect of irrigation and plant bio-regulators on seed yield, biological yield and harvest index of the Egyptian clover in Khajura, Banke, 2017/18

Factors	Seed yield, kg ha^{-1}	Biological yield, kg ha^{-1}	Harvest Index
Irrigation			
2-irrigations	834.63	8638	9.67
Zero irrigation	652.50	6444	10.13
Significance	**	**	*
% CV	7.62	13.67	10.23
Plant bio-regulators Spray			
2-sprays	911.31	6216	14.68
No spray	575.81	7869	7.32
Significance	**	NS	*
% CV	8.46	16.41	12.23

CV= Coefficient of variation

Likewise, the treatments of 2-sprays of plant bio-regulators had yielded better ($p<0.01$) seed yield than the treatments with no spray. But, both the treatments of 2-sprays and no spray of plant bio-regulators had yielded statistically similar ($p>0.05$) biological yield. In the case of harvest index, the treatments of 2-sprays of plant bio-regulator were better ($p<0.01$) compared to the treatments of no spray.

In the experiment, the treatment combinations of the irrigation and plant bio-regulators spray interactions were obtained non-significant for the parameters- seed yield, biological yield and harvest index.

The effect of the both factors were obtained significantly different for seed yield attributing characters, except the effect of plant bio-regulators spray on number of viable nodules (Table 2). The seed yield attributing characters were better for the treatments with 2-irrigations in comparison to zero irrigation treatments. Accordingly, number of inflorescence per m^2 were obtained significantly higher ($p<0.01$) for 2-irrigations in comparison to zero irrigation treatments. Similarly, number of florets per inflorescence and number of florets per plant were also significantly higher ($p<0.05$) for 2-irrigations treatments. Likely, number of viable nodules were also significantly larger ($p<0.01$) for 2-irrigations compared to zero irrigation.

The effect of plant bio-regulators spray on seed yield attributing characters of Egyptian clover is also presented in Table (2). The 2-spray of plant bio-regulators had resulted better number of inflorescence per m^{-2} ($p<0.01$), number of florets per inflorescence ($p<0.01$) and number of florets per plant ($p<0.01$). But, number of viable nodules per plant was not significantly affected ($p>0.05$) by the spray of plant bio-regulators (10.66±2.11 and 11.03±1.67 for 2-sprays and no spray treatments, respectively).

Table 2. Effect of irrigation and plant bio-regulator spray on the seed yield attributing characters of Egyptian clover in Khajura, Banke, 2017/18

Factors	Number of inflorescence m^{-2}	Number of florets $inflorescence^{-1}$	Number of viable nodule, $plant^{-1}$
Irrigation			
2-irrigations	1413.50	119.03	12.56
Zero irrigation	1291.81	84.46	9.12
Significance	**	*	**
% CV	17.13	17.17	17.66
Plant bio-regulators spray			
2-sprays	1510.31	109.26	10.66
No spray	1195.00	84.23	11.03
Significance	**	**	NS
% CV	8.63	7.05	19.36

CV= Coefficient of variation

The interactions of treatment combinations of irrigation and plant bio-regulators spray were significant for the number of inflorescence per m^2 (Table 3). The number of inflorescences per m^2 were higher ($p<0.05$) for the treatment of 2-irrigations with 2-spray of plant bio-regulators in comparison to the treatment of zero irrigation and no spray of plant bio-regulators. But, number of florets per inflorescence, number of florets per plant and number of viable nodules per plant were not significantly different ($p>0.05$) among the treatment combinations (Table 3).

Table 3. Effect of treatment combinations of irrigation and plant bio-regulators on seed yield attributing characters of Egyptian clover in Khajura, Banke, 2017/18

Treatments	Number of inflorescence m^{-2}	Number of florets $inflorescence^{-1}$	Number of viable nodule $plant^{-1}$
2-irrigations and 2-sprays	1594.50[a]	111.38	13.25
2-irrigations and no spray	1232.50[b]	86.77	9.44
Zero irrigation and 2-sprays	1426.12[ab]	107.21	8.81
Zero irrigation and no spray	1157.50[b]	81.71	11.88
Significance	*	NS	NS
%CV	10.04	8.34	9.43

Note: The mean values with different superscripts within the column are significantly different; CV= Coefficient of variation

Days taken to flower initiation, 50% flowering, 100% flowering and days to maturity for different treatment combinations are presented in Figure (1). The parameters such as days taken to flower initiation, 50% flowering, 100% flowering, and days to maturity remained statistically similar ($p>0.05$) among the treatments. The mean value for days taken to flower initiation ranged 140.12 to 152.69. Likely, the treatments mean of days to 50% flowering and 100% flowering ranged from 150.21 to 161.11 and 159.33 to 175.36 days after sowing, respectively. Similarly, the means for days to maturity for seed production was ranged from 170.07 to 179.02 days after sowing (Figure 1).

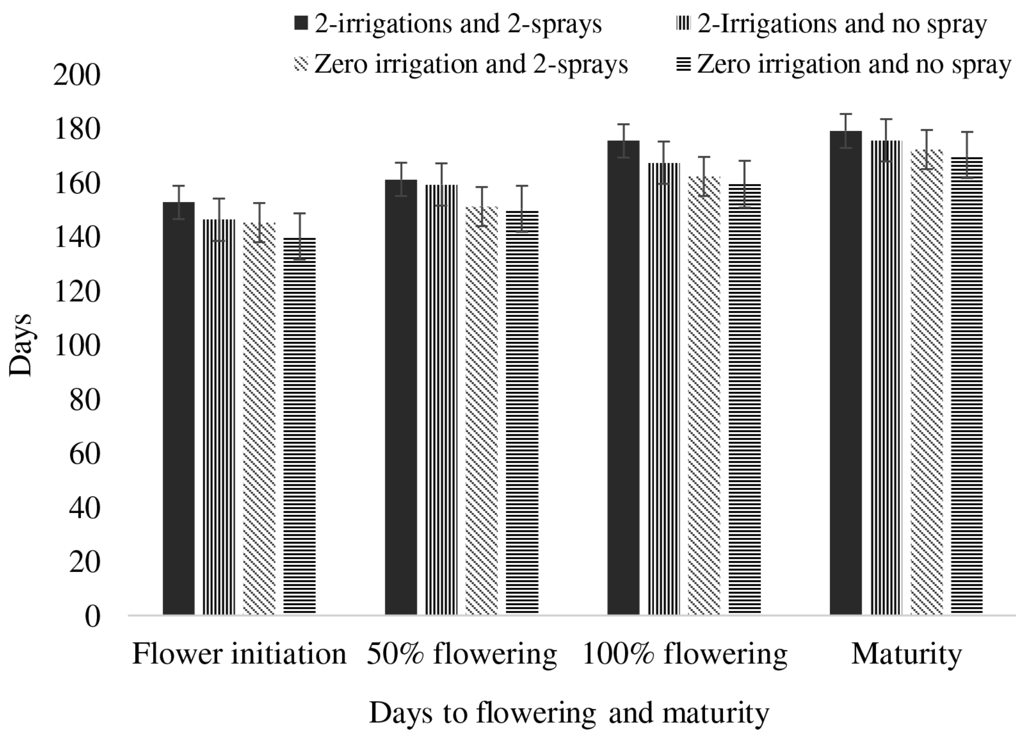

Figure 1. Days to flowering and maturity of Egyptian clover under different treatments

The germination percentage, test weight of 1000 seeds and seedling vigor index of seed produced under different treatments are presented in Table (4). The seeds produced from 2-irrigations treatments had better ($p<0.01$) germinations than the seeds from the zero irrigation treatments. Similar results were obtained in the case of the test weight of thousand seeds. The seedling vigor index was also better for the seeds from the 2-irrigations treatments compare to zero irrigation. Likewise, the treatment of plant bio-regulator spray had resulted higher germination percentage and test weight of seeds and better seedling vigor index in comparison to the no spray treatments.

Table 4. Effects of irrigation and plant bio-regulators spray on seed quality parameters of Egyptian clover in Khajura, Banke, 2017/18

Factors	Germination, %	TSW (g per 1000 seeds)	Vigor Index
Irrigation			
2-irrigations	92.94	4.23	5.94
Zero irrigation	81.19	3.25	4.80
Significance	**	*	*
% CV	5.79	5.45	14.82
Bio-regulator Spray			
2-sprays	91.38	4.38	6.27
No spray	81.75	3.11	4.08
Significance	*	**	**
% CV	6.50	4.58	5.36

CV= Coefficient of variation

The interactions of treatment combinations of irrigation and plant bio-regulators spray was significant ($p<0.05$) for seed quality parameters (Table 5). The seeds from the treatment combination of 2-irrigations and 2-spray of plant bio-regulators had significantly better ($p<0.05$) germination percentage than other treatments. The treatment combination with zero irrigation and no spray of plant bio-regulators resulted significantly lower ($p<0.05$) seed germination percentage. The similar results were obtained for test weight of 1000 seeds under different treatments. The seeds obtained from the treatment combination of 2-irrigations and 2-sprays of the plant bio-regulators were heaviest ($p<0.05$) for test weight, and the seeds of the treatment with zero irrigation and no spray of plant bio-regulator had resulted the lightest test weight. Likewise, the vigor indices of the seeds were best for the treatment combination of 2-irrigations and 2-spray of plant bio-regulators (Table 5).

Table 5. Treatment combinations interaction of irrigation and plant bio-regulators spray on seed quality parameters of Egyptian clover in Khajura, Banke, 2017/18

Factors		Germination, %	TSW, g per 1000 seeds	Vigor Index
2-irrigations	2-sprays	95.25^{a}	4.39^{a}	6.58^{a}
	No spray	86.62^{b}	3.67^{bc}	4.60^{c}
Zero irrigations	2-sprays	89.50^{b}	3.96^{ab}	5.35^{b}
	No spray	76.88^{c}	2.98^{c}	4.25^{c}
Significance		*	*	**
% CV		5.27	4.08	15.06

Note: The mean values with different superscripts within the column are significantly different; CV= Coefficient of variation

DISCUSSION

Egyptian clover is one of the most imperative fodder crops during the winter seasons in the dairy pocket areas of Nepal. In spite of growing popularity for fodder crops, farmers are not enthusiastic to produce its seed. Lesser yield and poorer quality of seed is one of the reasons of the farmers' reluctance to grow Egyptian clover for seed.

In most of the cases of Nepal, farmers who are cultivating Egyptian clover for seed production do not irrigate the seed crop after the cuttings for green fodder. While, irrigation is pivotal during lifecycle of the plants which helps in the nutrient uptake through xylem. Since the water regulates the biochemical and physiological functions in the plant, the sum water in the plants enriched with the soil nutrients maximizes the vegetative growth and seed yield (El-Bably, 2002). Seed yield of Egyptian clover could be enhanced by optimum irrigation scheduling after taking last cutting for fodder (Bakheit et al., 2012). Supporting to these reports, the results of the present study revealed that the seed yield and biological yield were significantly increased by two irrigations (at the rate of 666.67 kiloliters of water ha^{-1}) in 15 days interval after the cutting for green fodder at 60 days after sowing. The irrigation had improved the harvest index also in the experiment. The seed yield and biological yield were significantly lower for the Egyptian clover crop with zero irrigation treatments. In a previous study, Dost (1997) reported that the seed yield of Egyptian clover severely reduced with the decreased irrigation because of flower and head abscissions. When Egyptian clover is subjected to high temperature and water stress during seed setting and filling stage, it results in a significant reduction in seed yield and vigor (Iannucci et al.,1996). Application of irrigation increases the water productivity which resulted the more efficient utilization of the soil nutrients during the growth period of the Egyptian clover plants. Well nourishment strengthen the plants and give vigorous growth which resulted the increased biomass and the seed yield (Chandio, 2005). The results of this study matches well with these findings.

Seed attributing characters (number of inflorescence per m^2, number of florets per inflorescence, number of florets per plant and number of viable nodule per plant) were improved significantly by the application of two irrigations at the rate of 666.67 kiloliters of water ha^{-1} after the cutting for green fodder at 60 days after sowing in the present experiment. These yield attributing characters which are reduced under high temperature and moisture stress conditions have a direct and positive effect on seed yield. Insufficiency of water in formation of these characters negatively influences the proportion of dry matter partitioned to reproductive growth (Iannucci et al., 2000), and have been shown to reduce seed yields by up to 50% (Iannucci & Martiniello, 1998). The number of nodules were also found to be improved by increasing the irrigation frequencies in another study also (Nagre & Keshkar, 1990). The application of two irrigations at the rate of 666.67 kiloliters of water ha^{-1} after cutting for fodder at 60 days had improved the germination percentage, test weight of 1000 seeds and vigor index of seeds in the present study.

The range of temperature from 28°C to 32°C, the Relative Humidity of 45% to 55% with little differences in day and night temperatures and adequate sunshine hours was reported as the best environmental conditions in Giza, Egypt in order to obtain highest seed yield of Egyptian clover (El-Naby et al., 2012). El-Naby et al. (2012) further elaborated that higher temperature and lower humidity at reproductive period reduces viability of pollen and resulted low seed yield. During flowering high temperature stimulates respiration that reduces net photosynthesis (Wahid et al., 2007) and enhances physiological losses of pollinated flowers and increase embryo abortion in clover species (Iannucci & Martiniello, 1998; Iannucci, 2001). These abiotic stresses of high temperature can be managed by applying the plant bio-regulators, such as sodium benzoate, salicyclic acid, calcium chloride and potassium nitrate, which are able to induce long-term thermo tolerance (Beltrano et al., 1999; Wahid et al., 2007; Rab & Haq, 2012), and helps to increase flowering and pod formation in number of crops including clovers (Sarkar & Malik, 2001; Patil et al., 2005; Jhang et al., 2009). The result of the present study also shown that two foliar sprays of the plant bio-regulators (P_2O_5 @ 2 kg ha^{-1}) + KNO_3 @ 4 kg ha^{-1}) at the pre-flowering stage had improved the seed yield and harvest index, but had not shown the effect on the biological yield, which is in agreement with the previous findings.

All these seed attributing characters, except number of nodule per plant, were also significantly improved by the application of two foliar spray of plant bio-regulators in one week interval at pre-flowering stage in the present experiment. Potassium nitrate (KNO_3) is an osmoprotectant which could have played an important role in adaptation of cells to abiotic stress through their effect on water uptake, root growth, maintenance of turgour pressure and thereby could have help on normal functioning of plants as reported by several authors (Bardhan et al., 2007; Wahid et al., 2007; ; Rab & Haq, 2012). But, numbers of viable nodules per plant were not affected by the application of plant bio-regulators in the present study. Likely, the bio-regulators had also improved the germination percent, test weight of 1000 seeds and vigor index in the study.

The treatment combination of 2-irrigations with two sprays of plant bio-regulators had improved the germination percentage and test weight of produced seeds and seedling vigor index, which was significantly superior to the treatment of zero irrigation and no spray. The results revealed that the seed quality of the Egyptian clover can be improved by the combined application of two irrigations after cutting for fodder at 60 days plant age and two foliar spray of plant bio-regulators (P_2O_5 @ 2 kg ha^{-1}) + KNO_3 @ 4 kg ha^{-1}) at the pre-flowering stage.

Some interactive results from the previous studies for irrigation frequencies showed that increased irrigation maximizes yield because of the Egyptian clover's crop typology as a crop of irrigated track and less ability to tolerate water scarcity (El-Bably, 2002; Daneshnia et al., 2016). While, some author with their disagreement, stating that the Egyptian clover has ability to tolerate water deficiency by shading of root zone of the soil where from it is growing. It adjust drought spell by maintenance of hydration of tissue (Iannucci, 2000). The Egyptian clover requires 625-750 mm of total irrigation water, however, too frequent irrigation can retard the development of root and stem (Oushy, 2008) and the irrigation scheduling has a strong positive relation with seed yield (Ud-Din et al., 2014). Similarly, the appropriate frequency of bio-regulators spray results its judicious use to increase the seed yield and seed quality of Egyptian clover. Thus succeeding experiments on evaluating the irrigation frequencies and scheduling especially after the cutting for fodder and identifying the appropriate frequency for plant bio-regulators spray are important to consider.

CONCLUSION

Results from this study clearly revealed that irrigations along with foliar applications of plant bio-regulators improve the seed yield and seed quality of Egyptian clover. Providing two irrigations at the rate of 666.67 kiloliters of water ha^{-1} in 15 days interval after cutting at 60 days plant age for fodder increase the seed yield and improve the seed quality. Likely, two foliar sprays of plant bio-regulators (P_2O_5 @ 2 kg ha^{-1}) + KNO_3 @ 4 kg ha^{-1}) in one week interval at the pre-flowering stage also increase the seed yield and improves the seed quality. Adoption of these technologies can make substantial contributions in improving the seed production and seed quality of Egyptian clover which could help in mitigating the shortage of quality seed in the country. To further concretize these findings, detailed investigations on identifying the appropriate frequency for scheduling of the irrigation, and bio-regulators applications are suggested.

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