

## **BERSEEM CLOVER (*Trifolium alexandrinum* L.) SEED YIELD AND SEED QUALITY RESPONSE TO IRRIGATION FREQUENCY AND FOLIAR SPRAY OF PLANT BIO-REGULATORS**

**R.P. Ghimire<sup>1\*</sup>, N.R. Devkota<sup>2</sup>, D. Devkota<sup>2</sup> and M.P. Sharma<sup>2</sup>**

<sup>1</sup>Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal

<sup>2</sup>Agriculture and Forestry University, Rampur, Chitwan, Nepal

### **ABSTRACT**

The primary challenges in Berseem clover (*Trifolium alexandrinum* L.) seed production in Nepal are low productivity levels and substandard quality indicators. To address this issue, the experiments were conducted at the Regional Agricultural Research Station, Khajura, Banke, Nepal, and replicated in farmers' fields using a Randomized Complete Block Design from November 2018 to June 2019. Four irrigation schemes and three levels of foliar sprays of plant bio-regulators were used. The experiment result revealed that three and four irrigations were most suitable for achieving higher seed yield ( $p < 0.05$ ), resulting in 0.99 and 0.93 t ha<sup>-1</sup> at on-station and 0.87 and 0.85 t ha<sup>-1</sup> at on-farm, respectively. Similarly, two and three sprays of plant bio-regulators yielded better ( $p < 0.05$ ) seed yields of 0.89 and 0.94 t ha<sup>-1</sup> at on-station and 0.79 and 0.85 t ha<sup>-1</sup> at on-farm, respectively, without significant alteration on days taken to seed maturity and biological yield. The interactions between the levels of irrigation and plant bio-regulators spray were non-significant ( $p > 0.05$ ) for seed yield and biological yield. The experiments demonstrated that three irrigations @ 666.67 kiloliters of water ha<sup>-1</sup> at 15-day intervals after one cut at 60 days after sowing, and three foliar sprays of plant bio-regulators (P<sub>2</sub>O<sub>5</sub> @ 2 kg ha<sup>-1</sup> + KNO<sub>3</sub> @ 4 kg ha<sup>-1</sup>) at 7-day intervals in the pre-flowering stage, significantly enhanced the seed yield and quality of Berseem clover.

**Keywords:** Biological yield, Germination, Harvest index, Seedling vigor, Yield components

### **INTRODUCTION**

In the dairy pocket regions of Nepal, Berseem clover (*Trifolium alexandrinum* L.) is of considerable importance as a nutritious fodder source with a quality harvest

---

\* Corresponding author: ramghimire.narc@gmail.com

comprising about 20% crude protein during dry seasons. Widely adopted as the primary winter fodder legume in the Terai regions of Nepal, it has been integral to winter feeding strategies for ruminants in the region and certain lower hills for over four decades (NAFLQML, 2019).

Despite its pivotal role, challenges persist in terms of seed productivity and quality in its seed production pockets. At the farm level in the mid-western Terai of Nepal, where seed pocket areas are located (Dang, Banke, Bardiya, Sarlahi, Mahottari and Dhanusha districts), Berseem clover exhibits lower seed productivity (100-200 kg seed ha<sup>-1</sup>) compared to other countries, such as Turkey, where yields can reach up to 1000 kg ha<sup>-1</sup>. This untapped yield potential is attributed to the absence of region-specific technologies for seed production, including critical factors such as water management, weather stress coping strategies, varietal selection, sowing dates, and overall seed quality and plant protection measures (Pande, 2014; NAFLQML, 2019). As a consequence, there is a substantial reliance on seed imports from other nations (NAFLQML, 2019). The use of appropriate irrigation management and application of plant bio-regulators can significantly enhance the seed yield and quality of legume crops on a larger scale (Patil et al., 2005; Zhang et al., 2009). Improving the seed yield and quality of Berseem clover necessitates region-specific technological interventions, achievable through rigorous and targeted research in Nepal (Singh and Singh, 2019).

This approach could help meet the growing demand for quality Berseem clover seed in the country. Therefore, there is an urgent need for a comprehensive strategy to promote Berseem clover seed production by developing farmer-aligned technologies. To address this, the study aims to develop technologies that enhance agronomic options and improve the seed yield and quality of Berseem clover at on-station and on-farm.

## MATERIALS AND METHODS

The experiments were conducted at on-station (180 masl), and also at on-farm (156 masl) in Terai regions of Nepal. The on-station experiment was conducted at the research site of the Regional Agricultural Research Station (RARS), Khajura, Banke, and on-farm in the farmer's field at Mainapokhar (Badhaiyatal Rural Municipality-7) of Bardiya district, in the mid-western Terai of Nepal from November 2018 to June 2019. Meteorological data for the on-station study site is illustrated in Fig.1.

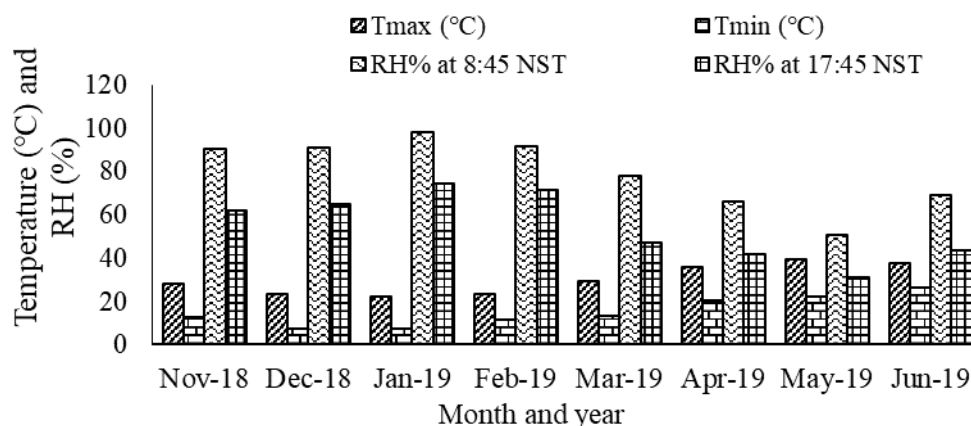


Figure 1. Monthly average temperature and relative humidity of experimental duration at RARS, Khajura, Banke

#### Experimental details and crop management

The experiments were laid out in Randomized Complete Block Design (RCBD) in a 4×3 factorial arrangement of two factors in both locations. The first-factor irrigation had four levels of frequencies of one, two, three and four irrigations @ 666.67 kiloliters of water ha<sup>-1</sup> at 15-day intervals after taking one cut at 60 DAS. Similarly, the second factor, plant bio-regulators application, had three frequencies of one, two and three foliar sprays of the plant bio-regulators (P<sub>2</sub>O<sub>5</sub> @ 2 kg ha<sup>-1</sup> + KNO<sub>3</sub> @ 4 kg ha<sup>-1</sup>) at the 7-day interval in the pre-flowering stage. Each treatment was replicated three times in every location. The plot size was 4×3 m<sup>2</sup> as an experimental unit.

The cultivar ‘Greengold’ was used in the experiments. The cultivation adhered to the crop management practices outlined by Vijay et al. (2017). The land underwent thorough tillage, and the dry sowing method was employed for field preparation. Fertilizers were applied as a basal dose @ 20:60:40 kg ha<sup>-1</sup> N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O during land preparation. Berseem clover seeds were sown using the line sowing method @ 20 kg seed ha<sup>-1</sup>, at a row-to-row distance of 40 cm and a seeding depth of 2-3 cm. Before sowing, seed inoculation with *Rhizobium leguminosarum* bv. *trifolii* was done. Regular surveillance and monitoring of diseases and insect pests were conducted, with hand weeding at 15 and 30 days after sowing (DAS) for weed management. A single cut for green fodder occurred at 60 DAS, after which the crop was left for seed production. Harvesting took place when the seeds within pods had turned yellow.

#### Field observations on seed yield and yield components

The determination of the date of flower initiation, 50% flowering, full flowering, and seed maturity, was conducted through visual observations following the methods outlined by Sardana and Narwal (2000). From each experimental plot, five inflorescence samples were taken from the harvested biological material, and the

number of florets per inflorescence was counted. The number of inflorescences  $m^{-2}$ , biological yield ( $t ha^{-1}$ ), seed yield ( $t ha^{-1}$ ), and harvest index (%) were calculated using the following equation and converted to  $t ha^{-1}$ .

$$\text{Number of inflorescence } m^{-2} = \frac{\text{Number of inflorescence in three central rows}}{\text{Row to row distance} \times \text{Row length} \times \text{Number of sample rows}}$$

$$\text{Biological yield, kg } m^{-2} = \frac{\text{Biological yield of three central rows, kg}}{\text{Row to row distance, m} \times \text{Row length, m} \times \text{Number of sample rows}}$$

$$\text{Seed yield, kg } m^{-2} = \frac{\text{Seed yield of three central rows, kg}}{\text{Row to row distance, m} \times \text{Row length, m} \times \text{Number of sample rows}}$$

$$\text{Harvest index, \%} = \frac{\text{Seed yield, t per ha}}{\text{Biological yield, t per ha}} \times 100$$

### Laboratory analysis

Seed samples were analyzed in the laboratory of National Seed Science Research Center, Khumaltar, Lalitpur. Test weight of 1000-seed was determined by Counting Replicates Method adhering to the International Rules for Seed Testing established by the International Seed Testing Association (ISTA, 2019). Seed purity, moisture content, other crop seeds, weed seeds and inert matters were analyzed. Then, determination of germination percentage, hard seed, fresh seed, abnormal seed, dead seed and seedling vigor indices I and II were done. The test procedures and computations followed the methodology of ISTA (1985); Krishnasamy and Seshu (1990) and Agrawal (1995).

### Statistical analysis

The data underwent statistical analysis using Analysis of Variance in the RCBD with two factors. Main and interactive effects were delineated. The data was analyzed by using the R-Package (R-Core Team, 2013). To differentiate between treatments at a 5% level of probability, Tukey's Honest Significant Difference (HSD) Test was employed.

## RESULTS AND DISCUSSION

### Effects on days taken to flowering and seed maturity

The results showed that the plants received three or four irrigation frequencies took significantly more days ( $p < 0.05$ ) to reach 50% flowering, full flowering, and seed maturity at both locations (Table 1). However, the effect of the number of sprays of plant bio-regulators was non-significant ( $p > 0.05$ ) on days taken to flowering and seed maturity at the research station and at the farmer's field as well. This delay in flowering initiation and seed maturation could be attributed to moisture stress or inadequate moisture levels, leading to reduced flowering vigor, as suggested by HSU (1994), especially in cases with fewer irrigation applications. At the research station and the farmer's field both, the interaction effects of various irrigation frequencies

and the number of plant bio-regulators sprays were found to be statistically non-significant ( $p>0.05$ ) for the duration taken to reach 50%-flowering, full flowering, and seed maturity.

Table 1. Effect of irrigation frequency and number of sprays of plant bio-regulators on days taken to flowerings and seed maturity of Berseem clover

Factors	Days to							
	Flower initiation		50%-flowering		Full flowering		Seed maturity	
	On-station	On-farm	On-station	On-Farm	On-station	On-farm	On-station	On-farm
Irrigation frequency								
One	-	-	121.75 <sup>c</sup>	127.00 <sup>b</sup>	137.50 <sup>c</sup>	143.50 <sup>b</sup>	178.50 <sup>b</sup>	183.75 <sup>b</sup>
Two	-	-	125.75 <sup>bc</sup>	133.25 <sup>ab</sup>	142.50 <sup>bc</sup>	146.25 <sup>ab</sup>	182.00 <sup>ab</sup>	187.00 <sup>ab</sup>
Three	-	-	132.00 <sup>ab</sup>	137.75 <sup>ab</sup>	151.25 <sup>ab</sup>	156.25 <sup>ab</sup>	187.25 <sup>a</sup>	192.25 <sup>ab</sup>
Four	-	-	136.25 <sup>a</sup>	143.00 <sup>a</sup>	153.75 <sup>a</sup>	161.75 <sup>a</sup>	187.90 <sup>a</sup>	195.00 <sup>a</sup>
SEM	-	-	3.26	3.38	3.71	4.27	2.23	2.53
Significance	-	-	*	*	*	*	*	*
LSD <sub>0.05</sub>	-	-	9.68	12.01	10.88	19.32	7.32	10.69
CV%	-	-	6.32	15.56	11.80	12.69	6.42	11.12
No of sprays of plant bio-regulators								
One	119.75	127.00	128.75	135.00	147.00	152.25	184.50	187.75
Two	119.20	127.25	128.25	133.75	145.25	150.75	182.75	189.75
Three	119.70	129.00	129.75	136.75	146.25	152.75	184.75	191.25
SEM	0.830	0.61	0.76	0.87	0.51	0.57	1.05	0.99
Significance	NS	NS	NS	NS	NS	NS	NS	NS
LSD <sub>0.05</sub>	2.40	3.29	2.66	4.89	2.06	2.61	4.89	5.39
CV%	9.71	12.29	6.32	15.56	11.86	12.69	6.42	11.12

Means bearing different superscripts in the same column differ significantly at a 5% probability level, SEM= Standard error of mean, LSD<sub>0.05</sub>= Least significant difference at 0.05, CV= Coefficient of variation, \*= Significant at a 5% level of significance, NS= non-significant.

### Effects on yield components

The results indicated that the increased irrigation frequencies (two, three, and four) and two and three sprays of plant bio-regulators had improved ( $p<0.05$ ) the number of inflorescences  $m^{-2}$  and number of florets inflorescence<sup>-1</sup> at on-station and at on-farm as well (Table 2). The test weight of 1000-seed was influenced by both factors, irrigation frequency ( $p<0.05$ ) and the number of plant bio-regulators spray ( $p<0.01$ ), indicating better results for three and four irrigations and three sprays of plant bio-regulators. Similarly, the use of plant bio-regulators, including KNO<sub>3</sub>, has demonstrated positive impacts on seed yield components in various crops (Kumar et al., 2013), supporting the results of this study.

Foliar sprays of plant bio-regulators, particularly potassium nitrate ( $\text{KNO}_3$ ), might have contributed to heavier test weights of 1000-seed. This enhancement could be attributed to improved osmotic turgor regulation, water balance management, increased chlorophyll content, enhanced enzyme activities, improved translocation of photosynthates, and better building of food reserves for elevated seed formations.

In addition, the spray of  $\text{KNO}_3$  might have positively influenced sugar accumulations in the collar regions of seed plants, resulting in better seed components, as supported by previous studies. The study aligns with the study of Mohammed and Fahmy (1988) that emphasizes the positive effects of plant bio-regulators on seed components of Berseem clover.

Table 2. Effects of irrigation frequency and number of plant bio-regulators spray on seed yield components of Berseem clover

Factors	Number of inflorescence $\text{m}^{-2}$		Number of florets inflorescence <sup>-1</sup>		Test weight of 1000-seed (g)	
	On-station	On-farm	On-station	On-farm	On-station	On-farm
Irrigation frequency						
One	1042.46 <sup>b</sup>	820.00 <sup>c</sup>	157.00 <sup>b</sup>	137.31 <sup>b</sup>	3.59 <sup>d</sup>	3.07 <sup>b</sup>
Two	1224.12 <sup>a</sup>	1003.11 <sup>b</sup>	210.67 <sup>ab</sup>	159.92 <sup>ab</sup>	4.13 <sup>c</sup>	3.62 <sup>ab</sup>
Three	1329.71 <sup>a</sup>	1137.45 <sup>ab</sup>	243.25 <sup>a</sup>	172.22 <sup>a</sup>	4.92 <sup>a</sup>	3.93 <sup>a</sup>
Four	1311.13 <sup>a</sup>	1174.28 <sup>a</sup>	229.33 <sup>a</sup>	177.47 <sup>a</sup>	4.47 <sup>b</sup>	3.90 <sup>a</sup>
SEM	65.61	80.09	18.86	9.01	0.057	0.19
Significance	*	***	***	*	***	*
LSD <sub>0.05</sub>	116.67	154.06	56.29	26.29	0.27	0.59
CV%	19.64	19.66	14.36	21.69	2.81	10.10
No of plant bio-regulators spray						
One	1093.69 <sup>b</sup>	877.79 <sup>b</sup>	147.25 <sup>b</sup>	132.64 <sup>b</sup>	3.92 <sup>c</sup>	3.10 <sup>b</sup>
Two	1254.20 <sup>a</sup>	1067.33 <sup>a</sup>	221.50 <sup>a</sup>	169.80 <sup>a</sup>	4.22 <sup>b</sup>	3.67 <sup>ab</sup>
Three	1334.06 <sup>a</sup>	1158.62 <sup>a</sup>	261.25 <sup>a</sup>	188.74 <sup>a</sup>	4.72 <sup>a</sup>	4.12 <sup>a</sup>
SEM	71.35	82.78	30.03	16.14	0.049	0.29
Significance	***	***	*	**	***	**
LSD <sub>0.05</sub>	146.00	174.62	76.62	35.69	0.24	0.85
CV%	19.00	19.66	14.08	21.69	2.81	10.10

Means bearing different superscripts in same column differ significantly at 5% level of probability, SEM= Standard Error of Mean, LSD<sub>0.05</sub>= Least Significant Difference at 0.05, CV= Coefficient of Variation, \* = Significant at 5% level of significance, \*\*= Significant at 1% level of significance, \*\*\*= Significant at 0.1% level of significance.

At the on-station, the interaction effects of higher irrigation frequency and two and three sprays of plant bio-regulators on the numbers of inflorescences  $\text{m}^{-2}$  ( $p < 0.01$ ),

florets inflorescence<sup>-1</sup> ( $p < 0.01$ ) and test weights of 1000-seed were significantly higher ( $p < 0.001$ ), whereas the interaction effect of these parameters were non-significant ( $p > 0.05$ ) at on-farm.

### **Effects on seed yield, biological yield and harvest index**

#### **Location effects**

Significant effects ( $p < 0.05$ ) of location on seed yield were evident in the combined analysis of data from both on-station and on-farm experiments. Likewise, the location effects were also significant ( $p < 0.01$ ) for biological yield and harvest index.

#### **Treatment effects**

The results from the on-station experiment in RARS, Khajura, Banke, revealed a significant enhancement ( $p < 0.05$ ) in the seed yield of Berseem clover with three and four irrigations compared to one irrigation (Table 3). However, the seed yields between three and four irrigations were statistically similar ( $p > 0.05$ ). Likewise, in the farmer's field, three and four irrigations produced significantly higher seed yields ( $p < 0.001$ ) compared to one or two irrigations. The combined analysis of data from both on-station and on-farm experiments revealed that the applications of three irrigations showed significantly higher ( $p < 0.05$ ) seed yield compared to one and two irrigations. Seed yield remained similar for four irrigations, suggesting a non-significant reduction ( $p > 0.05$ ) beyond three irrigations. The increased frequency of irrigation (three or four times) might have provided a continuous supply of soil moisture, fostering vigorous plant growth, as reported by Leghari (2016). The higher biological yields for three and four irrigations in the study further support this statement. Adequate moisture might have enhanced physiological processes such as photosynthesis, mineral uptake, and nutrient transmission in plants. Insufficient moisture, on the other hand, could have lower NPK concentration, disturbed hormone balance (e.g., cytokinin and, abscisic acid), and adversely affected seed and biological yield in Berseem clover.

Likewise, the combined analysis of biological yield data from on-station and on-farm experiments revealed similar results to average seed yield. Lower irrigation frequencies (one and two times) may have led to reduce stem and root growth, reflected in the stem and root length ratio, crown diameter, and number of branches of Berseem clover. This reduction could be attributed to moisture insufficiency, as suggested by Wafaa et al. (2014), resulting in decreased biological yield. The variations in biological yields due to different irrigation frequencies showed corresponding effects on seed yield during the experiments, highlighting a positive relationship between biological yield and seed yield (Amato et al., 2013; Tufail, 2016).

Table 3. Effect of irrigation frequency and number of plant bio-regulators spray on seed yield, biological yield and harvest index

Factors	Seed yield, t ha <sup>-1</sup>			Biological yield, t ha <sup>-1</sup>			Harvest index, %		
	On-station	On-farm	Average	On-station	On-farm	Average	On-station	On-farm	Average
Irrigation frequency									
One	0.712 <sup>c</sup>	0.614 <sup>b</sup>	0.663 <sup>c</sup>	7.989 <sup>b</sup>	9.31 <sup>c</sup>	8.65 <sup>b</sup>	10.39	7.20	8.61
Two	0.812 <sup>bc</sup>	0.726 <sup>b</sup>	0.799 <sup>b</sup>	8.511 <sup>b</sup>	10.44 <sup>bc</sup>	9.47 <sup>b</sup>	11.55	7.52	9.54
Three	0.993 <sup>a</sup>	0.875 <sup>a</sup>	0.929 <sup>a</sup>	9.656 <sup>a</sup>	11.75 <sup>ab</sup>	10.70 <sup>a</sup>	11.45	8.18	9.82
Four	0.931 <sup>ab</sup>	0.853 <sup>a</sup>	0.872 <sup>ab</sup>	9.878 <sup>a</sup>	12.72 <sup>a</sup>	11.30 <sup>a</sup>	9.96	7.37	8.67
SEM	0.053	0.059	0.047	0.192	0.83	0.5578	0.85	0.81	0.90
Significance	*	***	***	***	**	***	NS	NS	NS
LSD <sub>0.05</sub>	0.153	0.123	0.094	0.611	1.73	1.12	1.98	1.66	1.82
CV%	18.4	16.35	17.27	6.94	16.12	16.68	23.94	22.72	19.55
Numbers of plant bio-regulator spray									
One	0.761 <sup>b</sup>	0.661 <sup>b</sup>	0.726 <sup>b</sup>	9.042	10.59	9.82	9.26 <sup>b</sup>	7.12	8.18
Two	0.895 <sup>a</sup>	0.794 <sup>a</sup>	0.830 <sup>a</sup>	8.983	11.23	10.18	11.34 <sup>a</sup>	7.36	9.33
Three	0.938 <sup>a</sup>	0.846 <sup>a</sup>	0.892 <sup>a</sup>	9.000	11.34	10.17	11.68 <sup>a</sup>	8.23	9.95
SEM	0.057	0.512	0.041	0.332	0.72	0.4831	0.81	0.70	0.78
Significance	*	*	***	NS	NS	NS	*	NS	NS
LSD <sub>0.05</sub>	0.133	0.106	0.082	0.788	1.46	0.988	1.68	1.52	1.96
CV%	18.4	16.35	17.27	6.94	16.12	16.68	18.94	22.72	20.55

Means bearing different superscripts in the same column differ significantly at 5% level of probability, SEM= Standard Error of the Mean, LSD<sub>0.05</sub>= Least Significant Difference at 0.05, CV= Coefficient of Variation, \* = Significant at 5% level of significance, \*\*= Significant at 1% level of significance, \*\*\*= Significant at 0.1% level of significance, NS= non-significant

The treatments involving two and three applications of plant bio-regulators demonstrated significantly higher seed yields ( $p < 0.05$ ) compared to the treatment with only one spray. However, the seed yields under two and three sprays of plant bio-regulators were statistically similar ( $p > 0.05$ ) at both on-station and on-farm locations, as well as during the combined analysis of data (Table 3). The increased number of sprays of plant bio-regulators may have effectively mitigated high-temperature (Fig. 1) stress, as proposed by Kumar et al. (2013). The properties and actions of the sprayed plant bio-regulators, altering growth patterns and nutritional components, could have enhanced plant tolerance to heat stress during high temperatures of above 35°C from May to June in experimental sites (shown in Fig. 1). The synchronization of pollination and fertilization time with hot air temperature, low humidity, and hot desiccating winds can severely reduce seed settings in Berseem clover, as reported in several studies (El-Naby et al., 2011; El-Naby et al., 2012). Factors contributing to low seed setting may include pollen sterility, post-



fertilization abortion of developing seeds due to high temperature, and reduced pollen viability under high-temperature and low-humidity conditions (El-Naby et al., 2011; Vijay et al., 2017). The direct application of plant bio-regulators through foliar spray during pre-flowering stages might have been more effective in altering the life processes or structure of seed plants, improving seed yields, sizes, and quality, as reported by Nickell (1982). The significant improvement in seed yield might be associated with higher frequencies of foliar sprays of plant bio-regulators, and may be attributed to enhanced inflorescence densities, increased florets inflorescence<sup>-1</sup>, and heavier test weights obtained in the study (Table 2).

The plant bio-regulators, with phosphorus and potassium, could have played a vital role in energy production, osmotic adjustment, water uptake, root growth, and overall stress resistance in plants (Munir et al., 2004; Bardhan et al., 2007). Potassium nitrate, specifically, is known for its effectiveness in managing heat stress in plants. It aids in adaptation during abiotic stresses by influencing water uptake, root growth, and the maintenance of turgor pressure. The role of potassium in water relations, osmotic adjustment, stomatal movement, and plant stress resistance, as highlighted by Aown et al. (2012), likely contributed to the observed results. The consistent results between on-station and on-farm experiments, despite significant location effects ( $p < 0.05$ ), accentuate the consistency of the findings.

#### **Effects on seed quality**

The germination percentage and proportions of abnormal and dead seeds were unaffected ( $p > 0.05$ ) by irrigation frequencies. However, higher irrigation frequencies (three and four) significantly ( $p < 0.05$ ) improved the vigor index I and vigor index II at both on-station and on-farm locations (Table 4). Oushy (2008) also recommended irrigation during the bloom stage to achieve large-sized, high-quality Berseem clover seeds.

Similarly, three sprays of plant bio-regulators outperformed one or two sprays for germination percentages, vigor index I, and vigor index II significantly ( $p < 0.05$ ), without affecting proportions of abnormal and dead seeds in both on-station and on-farm experiments (Table 4). The size, weight, and density of crop seeds are influenced by cultivation practices and environmental conditions. The test weight of 1000-seed was higher with three sprays of plant bio-regulators, resulting the better vigor indices. Similar improvements in seed quality were reported by Kumar et al. (2013), who found better germination, shoot length, root length, and seedlings' vigor index with three foliar sprays of a 2% solution of  $KNO_3$  at a 7-day interval from flower initiation.

Table 4. Effect of irrigation frequency and number of plant bio-regulators spray on the seed quality parameters of Berseem clover

Factors	Germination, %		Abnormal seed, %		Dead seed, %		Vigor index I		Vigor index II	
	On-station	On-farm	On-station	On-farm	On-station	On-farm	On-station	On-Farm	On-station	On-farm
Irrigation frequency										
One	85.89 <sup>b</sup>	86.57 <sup>b</sup>	1.67	3.07	1.78	1.19	564.56 <sup>b</sup>	587.02 <sup>b</sup>	5.41 <sup>b</sup>	5.31 <sup>c</sup>
Two	89.78 <sup>b</sup>	89.98 <sup>b</sup>	1.67	2.73	2.11	1.39	591.02 <sup>b</sup>	607.83 <sup>b</sup>	5.58 <sup>b</sup>	5.82 <sup>b</sup>
Three	91.00 <sup>a</sup>	93.51 <sup>a</sup>	1.11	2.92	1.78	1.59	618.78 <sup>a</sup>	640.32 <sup>a</sup>	5.94 <sup>a</sup>	6.19 <sup>ab</sup>
Four	90.78 <sup>a</sup>	93.91 <sup>a</sup>	1.78	2.57	2.11	1.41	604.37 <sup>a</sup>	645.33 <sup>a</sup>	5.96 <sup>a</sup>	6.27 <sup>a</sup>
SEM	1.47	1.24	0.497	0.11	0.49	0.08	17.31	13.81	0.16	0.21
Significance	NS	NS	NS	NS	NS	NS	*	*	**	*
LSD <sub>0.05</sub>	3.04	9.67	0.982	1.21	0.63	0.56	35.89	52.22	0.33	0.47
CV%	3.48	13.69	27.76	10.76	23.65	8.61	6.17	12.32	5.81	7.63
No of sprays of plant bio-regulators										
One	85.75 <sup>c</sup>	87.49 <sup>b</sup>	1.49	3.11	1.83	1.37	566.55 <sup>b</sup>	584.15 <sup>b</sup>	5.50 <sup>b</sup>	5.52 <sup>b</sup>
Two	89.00 <sup>b</sup>	91.86 <sup>b</sup>	1.83	2.43	1.99	1.39	592.48 <sup>b</sup>	612.12 <sup>b</sup>	5.63 <sup>b</sup>	5.71 <sup>b</sup>
Three	93.33 <sup>a</sup>	96.38 <sup>a</sup>	1.33	2.93	1.99	1.42	625.01 <sup>a</sup>	654.22 <sup>a</sup>	6.04 <sup>a</sup>	6.26 <sup>a</sup>
SEM	1.27	2.59	0.430	0.21	0.42	0.014	14.99	20.22	0.14	0.21
Significance	***	*	NS	NS	NS	NS	**	*	**	*
LSD <sub>0.05</sub>	2.63	8.08	1.085	1.36	0.78	0.28	31.08	66.34	0.28	0.44
CV%	3.48	13.69	27.76	14.70	53.6	8.61	6.17	12.32	5.81	7.63

Means bearing different superscripts in the same column differ significantly at a 5% level of probability, SEM= Standard error of the mean, LSD<sub>0.05</sub>= Least significant difference at a 5% level, CV= Coefficient of variation, \* = Significant at a 5% level of significance, \*\*= Significant at 1% level of significance, \*\*\*= Significant at 0.1% level of significance, NS= non-significant

The interaction effects of irrigation frequencies and the number of plant bio-regulators spray showed non-significant interaction ( $p>0.05$ ) on germination percentages, percentages of abnormal and dead seeds, vigor index I, and vigor index II at both on-station and on-farm experiments.

The results from the study revealed that the application of three irrigations and three plant bio-regulator sprays emerges as a potential strategy to yield superior-quality Berseem clover seeds.

### CONCLUSION

The research findings demonstrated that the application of three irrigations @ 666.6 kiloliters of water ha<sup>-1</sup> at 15-day intervals after one cut at 60 DAS for fodder, and three foliar sprays of plant bio-regulators (P<sub>2</sub>O<sub>5</sub> @ 2 kg ha<sup>-1</sup> + KNO<sub>3</sub> @ 4 kg ha<sup>-1</sup>) at 7-day intervals during the pre-flowering stage significantly improves both seed yield and seed quality of Berseem clover in the mid-western Terai of Nepal. The non-significant interaction effects of irrigation frequencies and number of sprays of plant bio-regulators on seed yield and biological yield imply that the effects of irrigation and the number of bio-regulator sprays are largely independent. However, a combined analysis of key parameters, considering the significant impact of location, suggests that interactive effects may be location-specific and subject to variation.

### ACKNOWLEDGEMENT

The experiments formed an integral part of the PhD dissertation. The authors express sincere gratitude to the Agriculture and Forestry University, Rampur, Chitwan, Nepal, the Nepal Agricultural Research Council (NARC) and involved farmers. Additionally, special thanks are extended to the Agriculture and Food Security Project (AFSP), a collaboration between the World Bank and the Government of Nepal, for their generous financial support for this program.

### REFERENCES

- Agrawal, R.L. (1995). Seed testing. Seed Technology (2<sup>nd</sup> Edition). Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi. Pp. 437-626.
- Amato, G., Giambalvo, D. and Ruisi, P. (2013). Cut and post-cut herbage management affects Berseem clover seed yield. *Agronomy Journal*, 105: 1222-1230.
- Aown, M., Raza, S., Saleem, M.F., Anjum, S.A., Khaliq, T. and Wahid, M.A. (2012). Foliar application of potassium under water deficit conditions improved the growth and yield of wheat (*Triticum aestivum* L.). *Journal of Animal and Plant Sciences*, 2(22): 431-437.
- Bardhan, K., Kumar, V. and Dhimmsar, S.K. (2007). An evaluation of the potentiality of exogenous osmoprotectants mitigating water stress in chickpea. *Journal of Agricultural Science*, 3: 67-74.
- El-Naby, A., Zeinab, M. and Sakr, H.O. (2012). Influence of ecological factors on seed setting and fertility of five Egyptian clover (*Trifolium alexandrinum* L.) cultivars. *Asian Journal of Plant Science and Research*, 2(4): 388-395.
- El-Naby, A., Zeinab, M., Sakr, H.O. and Azab, M.M. (2011). The effect of temperature, humidity, and wind speed on seed setting and fertility of five Egyptian clover (*Trifolium alexandrinum* L.) Cultivars. *Journal of Plant Production*, Mansoura University, 2(3): 401-412.
- HSU. (1994). Forage Seed Production. ILCA Training Manual. International Livestock Center for Africa (ILCA), Addis Ababa, Ethiopia.

- ISTA. (1985). International rules for seed testing. *Seed Science and Technology* 13(2): 421-463.
- ISTA. (2019). Thousands seed weight (TSW) determination. International Rules for Seed Testing. International Seed Testing Association (ISTA), Zurichstr. 50, CH-8303 Bassersdorf, Switzerland.
- Krishnasamy, V. and Seshu, D.V. (1990). Phosphine fumigation influence on rice seed germination and vigor. *Crop Science*, 30: 28-35.
- Kumar, B., Yadvinder, S., Ram, H. and Sarlach, R.S. (2013). Enhancing seed yield and quality of Egyptian clover (*Trifolium alexandrinum* L.) with foliar application of bio-regulators. *Field Crops Research*, 146: 25-30. doi: 10.1016/j.fcr.2013.03.004.
- Leghari, S.J. (2016). Growth and yield performance of Berseem (*Trifolium alexandrinum* L.) under the impact of varying levels of NPK and irrigation. *M. Sc. Thesis*, Sindh Agriculture University, Tandojam.
- Mohammed, N.A., and Fahmy, H.M. (1988). Effect of Alar, Gibberellic Acid and Morphactin on Egyptian clover (*Trifolium alexandrinum* L.). Proceedings of the 3<sup>rd</sup> Conference on Agronomy, Kafr el-Sheikh, Egypt.
- Munir, I., Ranjha, M., Rehman, S., Mehdiand, M. and Mahmood, K. (2004). Effect of residual phosphorus on sorghum fodder in two different textured soils. *International Journal of Agriculture and Biology*, 6(6): 967-969.
- NAFLQML. (2019). Balance sheet of animal feed and forage seed of Nepal and impact study of forage mission program. National Animal Feed and Livestock Quality Management Laboratory, Hariharbhawan, Lalitpur.
- Nickell, L.P. (1982). Plant growth regulators: Agricultural uses. Berlin-Heidelberg-New York, New York.
- Oushy, H. (2008). Fact Sheet: Egyptian Clover. Afghanistan Water, Agriculture and Technology Transfer (AWATT) Program. College of Agricultural, Consumer and Environmental Sciences, New Mexico State University, USA. <https://dokumen.tips/download/link/clover-brochure.html>. Accessed on 28<sup>th</sup> March, 2021.
- Pande, R.S. (2014). Berseem (*Trifolium alexandrinum*) seed production in Nepal. In: Muhammod, Dost, Misri, Bimal, EL-Nahrawy, Mohamed, Khan, Sartaj and Serkan, Ates (Editors), Egyptian Clover (*Trifolium alexandrinum*), King of forage crops. Food and Agriculture for United Nations, Cairo. <http://www.fao.org/3/a-i3500e.pdf>. Accessed on 23 November, 2022. Pp. 91-94.
- Patil, S.N., Patil, R.B. and Suryawanshi, Y.B. (2005). Effect of foliar application of plant growth regulators and nutrients on seed yield and quality attributes of mung bean (*Vigna radiata* L. Wilczek). *Seed Research*, 33: 142-145.
- R-Core Team, (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.r-project.org/>. Accessed on 28 April 2020.
- Sardana, V. and Narwal, S. S. (2000). Influence of time of sowing and last cut for fodder on the fodder and seed yields of Egyptian clover. *Journal of Agricultural Science*, 134: 285-291.

- Singh, A. and Kang, J.S. (2004). Effect of agro techniques in seed production potential of new berseem cultivar BL 42. *Range Management and Agroforestry*, 25: 80-81.
- Singh, S.B. and Singh, N. (2019). Nepal livestock feed balance and strategies to address the feed deficit. *Journal of Agriculture and Forestry University*, 3:159-171. <https://www.afu.edu.np/>. Accessed on 6<sup>th</sup> January 2023.
- Tufail, M.S. (2016). Development of Berseem clover (*Trifolium alexandrinum* L.), village-based forage seed enterprises for the profitability and sustainability of smallholder farmers of Pakistan in mixed farming systems. PhD Thesis. Charles Sturt University, New South Wales, Australia. 266p.
- Vijay, D., Manjunatha, N., Maity, A., Kumar, S., Wasnik, V. K., Gupta, C. K., Yadav, V.K. and Ghosh, P.K. (2017). BERSEEM- Intricacies of Seed Production in India. Technical Bulletin. ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India. 47p.
- Wafaa, M.S., Abdel-Tawab, F.M., Fahmy, E.M., Rammah, M. and Belal, A.H. (2014). Biochemical genetic fingerprinting and effect of water stress on Egyptian clover (*Trifolium Alexandrinum* L.). In: Muhammod, Dost, Misri, Bimal, EL-Nahrawy, Mohamed, Khan, Sartaj and Serkan, Ates (Editors), Egyptian Clover (*Trifolium alexandrinum*), King of forage crops. Food and Agriculture for United Nations, Cairo. <http://www.fao.org/3/a-i3500e.pdf>. Accessed on 23 November, 2022. Pp. 53-54.
- Zhang, T., Wang, X., Wang, Y., Han, J. and Majurus, M. (2009). Plant growth regulator effects on balancing vegetative and reproductive phase in alfa-alfa seed yield. *Agronomy Journal*, 101: 1139-1145.

