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Research Article

Seed germination and seedling growth performance of berseem clover (*Trifolium alexandrinum* L.) populations under different irrigation water sources

Muhammad Shoaib Tufail[1,4], Gaye L. Krebs[2,4], Muhammad Shoaib Khan[1,5], Alison Southwell[4], John W. Piltz[3], Mark R. Norton[3,4] , Peter C. Wynn[4]

[1] *Department of Primary Industries and Regional Development, South Perth Western Australia 6151.*
[2] *School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Wagga Wagga New South Wales 2650.*
[3] *Wagga Wagga Agricultural Institute, Department of Primary Industries, Wagga Wagga New South Wales 2650.*
[4] *Gulbali Research Institute, Charles Sturt University, Wagga Wagga New South Wales 2650.*
[5] *School of Agriculture and Environment, The University of Western Australia, Crawley Western Australia 6009.*

ABSTRACT

Irrigation water quality is closely associated with seed germination, however, information on the effect of water quality on berseem seed germination and seedling establishment is deficient. The present study evaluated the effects of using different irrigation water quality (indicative of local field practices); including distilled water (as control), canal water, tube well water and a mixture of canal and tube well water in a 50:50 ratio on three populations of berseem clover viz. cv. Agaitti Berseem-2002 (AB-2002) and two local landraces exchanged between farmers (LBF1) or available from local markets (LBM1). Seed germination percentage (GP) ranged from 89.5% to 99.5%, while the emergence energy four days after sowing (EE4DAS) ranged from 42.7% to 77.6%. The use of tube well water alone or in combination with canal water produced the highest GP (98.5%), EE4DAS (77.6%), germination rate (GR; 3.5 %/day), emergence index (EI; 13.8), shoot length (40.7mm), root length (20.9mm), root to shoot ratio (0.48), seedling vigour index (5835), shoot fresh and dry weights (135.7mg and 6.5mg) and root fresh and dry weights (58.8mg and 1.6mg), respectively. Moreover, all the pair-combination treatments between the water source and measured variables of using tube well water showed a strong positive correlation with increasing seedling growth parameters. The results further demonstrated that the seed genotypes significantly affected ($P < 0.05$) the studied parameters, with the AB-2002 population overall performed better in most seed germination and emergence parameters. However, the LBF1 seed population was found to be more robust in seedling growth and development traits.

Keywords: Field irrigation practices, Saline water, Crop establishment, Local landraces, Fodder development.

Correspondence
Muhammad Shoaib Tufail
Shoaib.Tufail@dpird.wa.gov.au

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INTRODUCTION

Berseem clover (*Trifolium alexandrinum* L.) is an important forage legume renowned both for its high productivity and nutritional quality (Purseglove, 1974). It is widely adapted to a range of farming systems across various parts of the world.

However, its productivity varies across regions influenced by many genetic and agronomic factors including seed quality, soil fertility, irrigation, planting and harvest times (Tufail et al., 2020) as well as environmental factors such as temperature, humidity and rainfall (Abdel-Galil et al., 2008). Seed germination and seedling establishment have been identified as the key limiting factors that significantly impact crop establishment, thus influencing both forage and seed production of berseem clover (Deepak, 2010, Iannucci, 2006).

Uniform and rapid seed germination in berseem clover is crucial for early crop establishment, subsequently resulting in higher production. Berseem clover populations differ in their response to biotic and abiotic factors affecting germination and establishment (Dheeravathu et al., 2021), particularly the quality of irrigation water (Yadav et al., 2007). Salt stress is one of the major abiotic stresses that can reduce seed germination and seedling establishment and growth through limiting water availability (Khanduri et al., 2021), causing ion cyto-toxicity and osmotic stress, reducing enzyme activity (Ambede et al., 2012, Rouhi et al., 2012), as well as suppressing carbohydrate, protein and fatty acid synthesis resulting in lower leaf photosynthesis early in development (Ambede et al., 2012).

Deepak (2010) found that the use of low salinity irrigation water (<8 dS/m) delayed germination by up to 50%, while irrigation water of higher salinity (16-20 dS/m) reduced germination by as much as 88%. However, cultivars responded differently to various salinity levels. The biomass of roots and shoots decreased progressively from 3.1 and 4.0 g/pot to 0.2 and 0.4 g/pot representing a 95% and 91% reduction in biomass, respectively, with increasing salinity from 0.12 to 10 dS/m. Recently, Dheeravathu et al. (2021) reported similar adverse effects on seed germination and seedling growth parameters including shoot and root lengths, and the weights of both single and multi-cut cultivars grown under higher salinity conditions (EC ≥ 8-16 dS/m). In addition, soil salinity depressed soil microbial activity, including soil respiration and enzyme activities (30-80%) (Ghollarata and Raiesi, 2007).

In the central Punjab region of Pakistan, tube well irrigation water often contains high concentrations of salts resulting in moderate to high electrical conductivity (EC > 2.1 dS/m) compared to canal water, which is usually much less saline (EC < 0.5 dS/m) (Mehboob et al., 2011). Therefore, successful seed germination, seedling establishment and high forage production are more likely using canal water resources with lower salinity levels. Unfortunately, the available volume of canal irrigation water in Pakistan is insufficient (28.3 litres for 140 ha ≈ 4.9 L/ha) to support agriculture (Tufail et al., 2017) and has decreased significantly by about 8.4% annually from 142-million-acre feet (MAF) to 96 MAF (175 to 118 ML) in a decade between 2011-2021. Thus, tube well water is used as an alternative to overcome this shortage, with 28 million cusec (68.5 million ML) pumped annually (Government of Pakistan, 2023). Despite 70-95% of the available tube well water being brackish in nature (Ghulam et al., 2013, Mehboob et al., 2011), it can be used effectively for irrigation with careful management where a strategy is developed for utilizing both canal and tube well water sources together. Applying alternating sources of canal and tube well waters (a common local farmer's practice) is one method used to minimize the adverse effects of low-quality irrigation water. Moreover, Ghulam et al. (2013) reported that using a mix of tube well water and canal water combined with gypsum also reclaimed saline-sodic soils and produced significantly higher forage yields of berseem clover and sorghum crops. In addition, different populations of berseem clover are known to differ in their response to varying salinity levels of irrigation water with some more tolerant than others (Deepak et al., 2010).

The impact of different irrigation water sources on berseem seed germination and seedling establishment has not been reported in Pakistan. The objective of the present study was to investigate the effects of different sources of irrigation water available to farmers on seed germination percentage, germination rate, shoot and root growth and development, and seedling vigour of 3 different berseem clover populations.

MATERIALS AND METHODS

Experimental Design

The study was conducted using a randomised block design with a 3 x 4 factorial arrangement in four replications during 2014-2015 at the field-testing laboratory of the University of Veterinary and Animal Sciences (UVAS), Ravi-campus, Pattoki. The treatment variables included three populations of berseem clover seed and four sources of irrigation water. Seed populations were obtained from local landraces exchanged between farmers or available from local markets and from a research-station *viz.* landrace berseem farmer own-saved seed retained on-farm (LBF1), landrace berseem seed sold locally in the agricultural market (LBM1) and Agaitti Berseem-2002 (AB-2002) which was a research-station bred improved cultivar obtained from the Fodder Research Institute, Sargodha-Pakistan. The irrigation water sources consisted of (1) Balloki Sulemanki Link Canal (BSLC) water (CW) extracted from the Rakh Distributary, (2) tube well water (TW) pumped at a depth of 100m from UVAS research farm Pattoki (31°3'40"N, 73°52'40"E), district Kasur of

Punjab-Pakistan, (3) a 50:50 mix of the above canal and tube well water (CTW), and (4) distilled water (DW; control). The water from all the irrigation sources was only collected once at the start of the trial and was the only water used throughout the trial. The three berseem clover populations were selected based on their agronomic performance, resilience to local environmental conditions, availability, and farmer preference. This selection allows for a comparative analysis between traditional farmer-saved and improved variety seeds to evaluate the impact of different irrigation water sources on seed germination and seedling establishment.

Chemical characteristics of the irrigation water sources

The chemical characteristics of the irrigation waters sourced from canal (CW), tube well (TW) and mixture of canal and tube well (CTW; 50:50 ratio) are presented in Table 1. There was a five-fold increase in salinity between the canal water (0.33 dS/m) and the tube well (1.63 dS/m), with the 50:50 canal + tube well water between the two (0.74 dS/m), as expected. Salinity levels for berseem clover production were low in the canal water, ranging to above the recommended level of 1.5 dS/m in the tube well (Prince, 2015). Further, sodium adsorption ratio (SAR) values above 10 mmol/L and residual sodium carbonate (RSC) levels greater than 2.5 me/L are considered to be brackish and unfit for use for irrigation purposes in the current cropping systems of central Punjab, Pakistan (Mehboob et al., 2011). SAR values of the tubewell water were approaching to an unsafe level whereas RSC would classify the water as unsuitable for irrigation.

Table 1**.** Quality of canal (CT), tube well (TW), and mixture of canal and tube well waters (CTW; 50:50 mixing ratio) used for growing *Trifolium alexandrinum* seeds**.**

Characteristics	Canal Water (CW)	Tube well Water (TW)	Canal + Tubewell Water (CTW; 50:50 mix)	Mean (±SED)
EC				
− dS/m	0.33	1.63	0.74	0.9 (±0.4)
− ppm	211	1040	474	575 (±245)
SAR (mmol/L)	0.7	9.8	1.2	3.9 (±2.9)
RSC (me/L)	0.5	3.5	0.2	1.4 (±1.0)
Ca + Mg (me/L)	2.5	3.5	5.3	3.8 (±0.8)
Na (me/L)	0.8	12.7	2.1	5.2 (±3.8)
HCO3 (me/L)	3.0	7.0	3.0	4.3 (±1.3)

EC: Electrical conductivity; SAR: Sodium adsorption ratio; RSC: Residual sodium carbonate
Parentheses indicate SED values; SED: Standard error of difference.

Experimental Trial Layout

The 12 treatments were randomly allocated to the 12 germination trays in each of the four replications with sand used as the germination medium. The trays were placed on a laboratory bench adjacent to a window which was the source of natural light; the temperature in the laboratory ranged from 22-26°C. As seed size varied between the populations, 50 seeds of uniform size for each population were selected with any seeds not properly formed being discarded. Seeds were placed on top of the sand and watered daily to saturation with their respective water source. The trays of seeds were observed daily, and the number of germinated seeds recorded on daily basis to enable calculation of the germination percentage (GP), germination rate (GR), emergence index (EI) and emergence energy 4 days after sowing (EE4DAS) (Rouhi et al., 2010). As the seeds were germinated on top of a sand medium, germination could be determined at a very early stage when the radicle growth exceeded 1 mm.

Ten germinated seeds were marked with toothpicks at the first day of emergence to evaluate seedling growth and development. After 2 weeks of growth, ten seedlings were carefully removed (ensuring all loose soil was removed) and the total seedling length, and root and shoot lengths (after cutting at the crown-point) were measured. The seedling vigour index was then calculated. The fresh weights of the shoots and roots were determined immediately after removing the seedlings from the planting medium using a digital balance (SHIMADZU/Model AUW120D). Dry weights were determined after oven drying (Memmert Beschickung/Model 100-800) at 70^{o}C for 48h (Shabbir et al., 2014).

Calculations

The germination percentage (GP) was determined according to the following equation:

$$GP\ (\%) = \frac{Gn}{N} \times 100$$

Where;

Gn = total number of seeds germinated after 2 weeks,

N = total number of sown seeds

The germination rate (GR) was determined using the formula of Quddus et al. (2014):

$$GR\ (\%/day) = \frac{G1/t1\ +\ G2/t2\ +\ G3/t3\ +\ \cdots\cdots\ +\ G14/t14}{Gfinal}$$

Where;

G = number of germinated seeds on a particular day,

t = number of days into the germination period,

Gfinal = total number of seeds germinated after 2 weeks

The energy of emergence four days after sowing (EE4DAS) was determined according to the equation below, as described by Shabbir et al. (2014) and Basra et al. (2005).

$$EE\ 4DAS\ (\%) = \frac{SLn\ 4DAS}{N} \times 100$$

Where;

SL_n *4DAS* = number of seedlings emerged four days after sowing,

N = total number of seeds sown

The emergence index (EI) was determined to quantify the germination expression that relates the daily germination rate to the maximum germination value according to Shabbir et al. (2014):

$$EI = \frac{T\ \times Gn}{N}$$

Where;

T = time period (number of days after sowing)

Gn = total number of seeds germinated

N = total number of seeds sown

The seedling vigour index (SVI) was calculated using the formula of Shabbir et al. (2014) and Iannucci (2006):

$$SVI = SL\ Length\ (mm)\ \times\ GP$$

Where;

SL = seedling length (mm)

GP = germination percentage

The *root-shoot ratio (RSR)* was calculated using the following formula:

$$RSR = \frac{DW\ root\ (mg)}{DW\ shoot\ (mg)}$$

Where;

DW root = dry weights of roots (mg)

DW shoot = dry weights of shoots (mg)

Statistical Analysis

The data for all parameters were statistically analysed using the linear mixed model (ASREML) procedure in GenStat® (17th edition) software (VSN International, 2014). The three seed populations (LBF1, LBM1 and AB-2002) and four irrigation water sources (DW, CW, TW and CTW) were identified as fixed effects, while the germination trays and replications were fitted as random effects in the statistical model. This enabled identification of the extent of interactions between seed populations and the irrigation water source treatments to be assessed. Multiple sampling in each tray of all parameters was performed to improve measurement precision. The least significant difference test at 5% probability level (P < 0.05) was used to compare treatment means. Further, Pearson's correlation matrix analysis was also performed using R (version 4.1.1) with the "psych" package (R Core Team, 2021) to determine the interrelationship between seedling growth variables and source of irrigation water.

RESULTS

Effects on Seed Germination and Emergence

The effects of irrigation water source and seed population on germination and emergence of berseem clover are presented in Table 2. There was a significant ($P < 0.05$) interaction between water source and seed population which showed effects on overall germination percentage and germination rate per day, producing 95.9% and 3.4 %/day, respectively. Independently, neither seed population (SS) nor irrigation water source (WS) had a significant effect ($P > 0.05$) on germination percentage or germination rate; however, there were significant interactions ($P < 0.05$) between water source and seed population (WS x SS) for both parameters.

Table 1. Effect of different sources of irrigation water and seed populations on seed germination and emergence attributes of *Trifolium alexandrinum* seeds.

Parameters	LBF1				LBM1				AB-2002				P value		
	DW	CW	TW	CTW	DW	CW	TW	CTW	DW	CW	TW	CTW	WS	SS	WS x SS (±SED)
GP (%)	97.5^{cd}	95.0^{bcd}	96.0^{bcd}	97.0^{bcd}	97.5^{cd}	89.5^{a}	98.5^{d}	97.5^{cd}	99.5^{d}	98.0^{d}	93.0^{abc}	92.5^{ab}	0.069	0.848	0.007 (±2.51)
GR (%/day)	3.48^{cd}	3.39^{bcd}	3.43^{bcd}	3.46^{bcd}	3.48^{cd}	3.19^{a}	3.52^{d}	3.48^{cd}	3.55^{d}	3.50^{d}	3.32^{abc}	3.30^{ab}	0.069	0.848	0.007 (±0.09)
EE4DAS (%)	75.9^{c}	77.6^{c}	70.3^{c}	65.4^{bc}	73.3^{c}	45.0^{a}	77.6^{c}	63.1^{bc}	68.8^{bc}	54.6^{ab}	74.1^{c}	42.7^{a}	<0.001	0.008	0.006 (±7.41)
EI	13.6^{c}	13.3^{bc}	13.4^{bc}	13.6^{bc}	13.6^{c}	12.5^{a}	13.8^{c}	13.6^{c}	13.9^{c}	13.7^{bc}	13.0^{ab}	12.9^{ab}	0.069	0.848	0.007 (±0.35)

GP: Germination percentage; GR: Growth rate, EE4DAS: Emergence energy four days after sowing; EI: Emergence index; LBF1: Landrace berseem farmer own-saved seed; LBM1: Landrace berseem market seed; AB-2002: Agaitti Berseem-2002 (improved variety)
CW: Canal water; TW: Tube well water; CTW: Canal and tube well water (50:50 ratio); DW: Distilled water; WS: Water sources; SS: Seed populations
SED: Standard error of difference; Parentheses indicate SED value
Values within rows with varying superscripts differ significantly ($P < 0.05$).

The germination percentage and germination rates for the canal water with the farmer bought-market seed (CW x LBM1) treatment were significantly lower ($P < 0.05$) than all other treatments except canal and tube well water with Agaitti Berseem-2002 (CTW x AB-2002) and tube well water also with Agaitti Berseem cultivar (TW x AB-2002). The tube well irrigation water increased overall germination percentage across all seed populations (Figure 1), however, the local farmer landrace (LBF1) was found to be robust when irrigated with canal water (farmers' usual practice for irrigation).

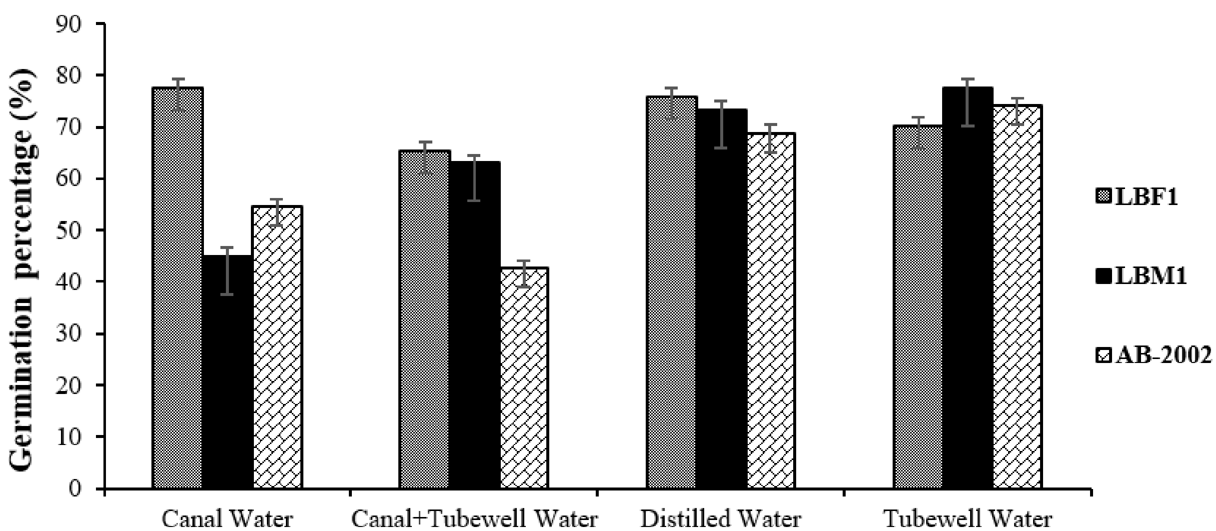

Figure 1. Effect of canal (CW), canal and tube well (CTW; 50:50 ratio), distilled (DW) and tube well (TW) sources of irrigation water on *Trifolium alexandrinum* seed germination percentages (%) of farmer own-saved (LBF1), market (LBM1) and Agaitti-Berseem-2002 (AB-2002) seed populations.

The seeds began to germinate within two days of sowing. Overall, the mean of EE4DAS was 65.7%. The EE4DAS of 50:50 mixture of canal and tube well water with Agaitti Berseem-2002 (CTW x AB-2002) and canal water with market seed (CW x LBM1) were significantly lower (*P* < 0.05) than all other treatments except canal water only with the Agaitti Berseem-2002 (CW x AB-2002) treatment. This did not differ (*P* > 0.05) from the 50:50 mixture of canal and tube well water treatments from other seed populations or distilled water with Agaitti-Berseem-2002 (DW x AB-2002). Independently, both water source and seed population had a significant effect (*P* < 0.05) on EE4DAS (Table 2). In addition, there was a significant interaction (*P* < 0.05) between water source and seed population on EE4DAS. Based on seed population, the means for EE4DAS were 72.3, 64.7 and 60% for LBF1, LBM1 and AB-2002, respectively. Based on water source, the mean EE4DAS were 72.7, 59.1, 73.9 and 57% for distilled water (DW), canal water (CW), tubewell water (TW) and the 50:50 mixture (CTW), respectively (Table 2). Further analysis of the data revealed that the EE4DAS was significantly higher (*P* < 0.001) when irrigated with tube well water (TW) as compared to canal (CW), and the 50:50 canal and tube well (CTW) waters across all the seed populations (Figure 2).

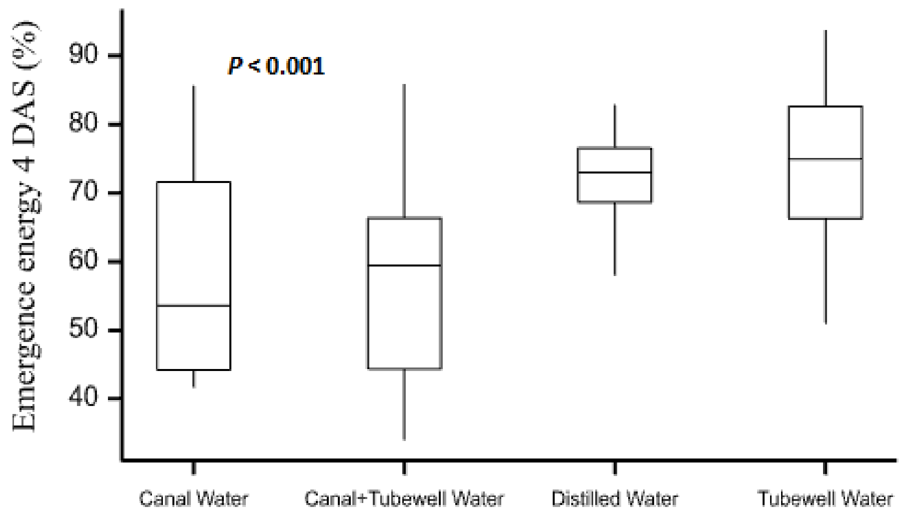

Figure 2. A boxplot shows significant effects (P < 0.001) of canal (CW), canal and tube well (CTW; 50:50 ratio), distilled (DW) and tube well (TW) sources of irrigation water on energy of emergence four days after sowing (EE4DAS; %) on *Trifolium alexandrinum* seed.

As with previous observations, the emergence index of canal water with market seed (CW x LBM1) was significantly lower (*P* < 0.05) than all other treatments except the 50:50 canal and tube well water with Agaitti Berseem-2002 (CTW x AB-2002) and tube well water with Agaitti Berseem-2002 (TW x AB-2002). Independently, neither seed population nor water source had a significant effect (*P* > 0.05) on emergence index; however, the interaction between water source and seed population (WS x SS) was significant (*P* < 0.05). The overall predicted mean of the emergence index was 13.43 (Table 2).

Effects on Seedling Growth and Development

The effects of irrigation water source on berseem seedling growth and development are presented in Table 3. Overall, the mean shoot length was 35 mm. The shoot length of 50:50 mixture of canal and tube well water treatments with Agaitti-Berseem-2002 (CTW x AB-2002; 22.6 mm) was significantly shorter (*P* < 0.05) than all other treatments except canal water with market seed (CW x LBM1; 26.7 mm) and distilled water with market seed (DW x LBM1; 29.4 mm). Only seed populations had a significant effect (*P* < 0.05) on shoot length although there was a significant interaction (*P* < 0.05) between water source and seed population on shoot length. Based on seed population, the predicted mean shoot lengths were 38.9, 32.6 and 33.6 mm for LBF1, LBM1 and AB-2002, respectively. The overall mean of root length was 18.2 mm. The root length of canal water with market seed (CW x LBM1; 14.4 mm) was significantly shorter (*P* < 0.05) than all other treatments except canal and tube well water with Agaitti-Berseem-2002 (CTW x AB-2002; 15.4 mm) and distilled water with market seed (DW x LBM1; 16.9 mm) which in turn did not differ (*P* > 0.05) from the remaining treatments. Again, only seed population had a significant effect (*P* < 0.05) on root length, with predicted mean root lengths of 19.5, 17.1 and 18 mm for LBF1, LBM1 and AB-2002, respectively (Table 3).

Results for seedling length mirrored that of shoot length. Both seed population as well as the interaction between water source and seed population had a significant effect (*P* < 0.05) on seedling length. The mean seedling lengths were predicted at 58.4, 49.7 and 51.6 mm for LBF1, LBM1 and AB-2002, respectively. The seedling length when using canal and tube well water with Agaitti-Berseem-2002 (CTW x AB-2002; 37.9 mm) was significantly shorter ($P < 0.05$) than all other treatments except canal water with market seed (CW x LBM1; 41.2 mm) and distilled water with market seed (DW x LBM1; 46.3 mm) as shown in Table 3. The overall mean of seedling length was 53.2 mm.

The predicted mean for fresh shoot weight over all treatments was 121.4 mg. The interaction between water source and seed population had a significant effect ($P < 0.05$), but independently, water source and seed population were not significant ($P > 0.05$). As with other growth parameters above, the fresh shoot weights of treatment canal and tube well water with Agaitti-Berseem-2002 (CTW x AB-2002) and canal water with market seed (CW x LBM1) were significantly less ($P < 0.05$) than most of the other treatments but not significantly less ($P > 0.05$) than canal and tube well water with farmer seed (CTW x LBF1) or distilled water with farmer seed (DW x LBF1).

Table 2. Effect of different sources of irrigation water and seed population on seedling growth and development attributes of *Trifolium alexandrinum*.

Parameters	LBF1				LBM1				AB-2002				*P* value		
	DW	CW	TW	CTW	DW	CW	TW	CTW	DW	CW	TW	CTW	WS	SS	WS x SS (±SED)
Shoot length (mm)	38.9^{c}	40.8^{c}	34.8^{bc}	40.7^{c}	29.4^{ab}	26.7^{a}	35.1^{bc}	39.3^{c}	39.1^{c}	35.3^{bc}	37.6^{c}	22.6^{a}	0.78	0.005	<0.001 (± 3.5)
Root length (mm)	20.2^{bc}	18.9^{b}	20.2^{bc}	18.9^{b}	16.9^{ab}	14.4^{a}	17.8^{b}	18.9^{b}	17.9^{b}	17.7^{b}	20.9^{bc}	15.4^{ab}	0.13	0.043	0.187 (± 1.8)
Seedling length (mm)	59.0^{cb}	59.8^{bc}	55.1^{b}	59.7^{bc}	46.3^{a}	41.2^{a}	52.9^{b}	58.2^{bc}	57.2^{bc}	52.9^{b}	58.3^{bc}	37.9^{a}	0.45	0.004	<0.001 (± 4.7)
Shoot fresh weight (mg)	120.4^{a}	127.0^{b}	123.9^{b}	115.0^{a}	134.2^{bc}	91.7^{ab}	119.9^{a}	135.7^{bc}	137.2^{bc}	142.5^{bc}	126.4^{b}	83.3^{a}	0.98	0.432	0.048 (± 20.1)
Shoot dry weight (mg)	4.95	5.52	4.50	6.05	4.67	4.40	5.10	4.70	5.70	5.70	6.50	3.02	0.70	0.802	0.315 (± 1.4)
Root fresh weight (mg)	46.2	30.5	58.8	40.7	46.4	46.5	52.9	48.9	61.7	45.7	56.6	39.6	0.15	0.559	0.731 (± 12.8)
Root dry weight (mg)	1.27	1.22	1.57	1.37	1.32	1.10	1.40	1.42	1.55	1.42	1.42	1.35	0.09	0.236	0.259 (± 0.1)
Root to shoot ratio	0.27	0.29	0.39	0.28	0.29	0.28	0.30	0.35	0.29	0.26	0.23	0.48	0.24	0.997	0.201 (± 0.1)
Seedling vigour index	5694^{bc}	5693^{bc}	5322^{bc}	5835^{bcd}	4544^{b}	3748^{a}	5244^{bc}	5674^{bc}	5753^{bcd}	5182^{bc}	5364^{bc}	3529^{a}	0.37	0.009	<0.001 (± 523)

LBF1: Landrace berseem farmer own-saved seed; LBM1: Landrace berseem market seed; AB-2002: Agaitti Berseem-2002
CW: Canal water; TW: Tube well water; CTW: Canal and tube well water (50:50 ratio); DW: Distilled water; WS: Water sources; SS: Seed populations
SED: Standard error of difference; Parentheses indicate SED value
Values within rows with varying superscripts differ significantly ($P < 0.05$).

As shown in Table 3, neither water source nor seed population, or the interaction between them had an effect ($P > 0.05$) on the dry weight of shoots, fresh weight of roots, dry weight of roots or the root to shoot ratio. The predicted mean of weight of fresh roots, dry roots and dry shoots was 47.9, 1.4 and 5.1 mg, respectively. Both seed population and the interaction between water source and seed population had a significant effect ($P < 0.05$) on SVI. The mean SVI was 5636, 4802 and 4957 for LBF1, LBM1 and AB-2002, respectively. The SVI for treatments canal and tube well water with Agaitti-Berseem-2002 (CTW x AB-2002) and canal water with market seed (CW x LBM1) were significantly lower ($P < 0.05$) than all other treatments (Table 3).

Pearson's correlation matrix further indicated that all the pair-combination treatments had a strong increasing relationship with no adverse effect. Shoot and root lengths as well as their fresh and dry weights correlated positively with the EC values or applied saline levels found in the tube well irrigation water (in this study). In particular, shoot length and shoot fresh weight showed highly positive correlations. Overall, a positive correlation was found among the studied attributes of seedling growth and development, and there was no negative interaction recorded with the use of tube well water (Figure 3).

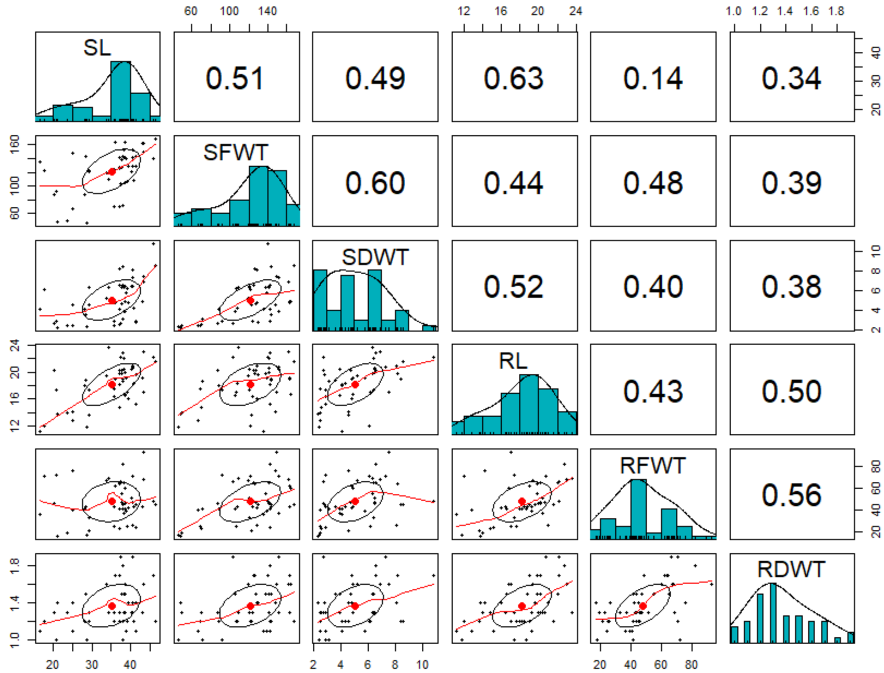

Figure 3. Pearson's correlation matrix between shoot length (SL), shoot fresh weight (SFWT), shoot dry weight (SDWT), root length (RL), root fresh weight (RFWT) and root dry weight (RDWT) of *Trifolium alexandrinum* seedlings resulting from the use of tube well (TW) irrigation water.

DISCUSSION

Impact on Seed Germination and Emergence

Rapid and uniform seed germination is most important for successful berseem crop establishment (Rouhi et al., 2010). Germination percentages were high, being close to or above 90% for all treatments. These results are consistent with Dheeravathu et al. (2021) and Deepak et al. (2010), who reported germination percentages of greater than 90% using irrigation water having an EC of ≤8 dS/m. Nichols et al. (2008) also reported that berseem clover seeds germinated well using water of this EC value. EC levels for all water sources used in the present study were well below this threshold. Some variation in germination and emergence parameters was observed across the treatments and the interaction between seed population and water sources was significant ($P < 0.05$) throughout, implying that the choice of the combination of berseem populations and irrigation water source can influence crop establishment outcomes. One would assume that seed sourced from local breeding programs (Agaitti-Berseem-2002; AB-2002) combined with better quality water (e.g., distilled and canal waters) would better promote germination and emergence. However, this was not observed in this study, rather the farmer grown landrace (LBF1) seed was more vigorous in both germination and emergence.

When using saline irrigation waters, excessive salt concentrations may affect seed germination by decreasing the rate and total amount of water absorbed and also by increasing the entry of chloride ions which may be toxic (Khatib and Massengale, 1966). Ambede et al. (2012) attributed this to the lower osmotic potential which reduces the availability of water for seed absorption, thus creating a salt-stimulated physiological drought stress. Deepak et al. (2010) reported that water with lower EC levels (of 4-8 dS/m) delayed germination, whereas water with higher levels (16-24 dS/m) greatly reduced the germination percentage. Conversely, the findings in this study agree with those of several authors who reported that low salt concentrations (up to 4 dS/m) may positively influence seed germination and thus increase germination percentages (Deepak et al., 2010, Dheeravathu et al., 2021).

Khatib and Massengale (1966) found that germination was stimulated at low salt concentrations (2000 ppm). Similarly, Rouhi et al. (2012), in a study conducted in Iran, found germination started quickly in the presence of salts (such as NaCl), and significantly increased ($P < 0.05$) overall germination percentage by 30%. More recently, Khanduri et al. (2021) have found that a salt concentration of up to 50mM (2300 ppm) had shown no major effects on germination rate of berseem clover seeds. These authors suggested that this may be due to the antagonistic phenomenon exhibited when cations mixed together cancel the toxic effects of individual salts on the permeability of the cytoplasmic membrane. Rouhi et al. (2012) also reported that the presence of NaCl in irrigation water increased both germination percentage and germination rate, potentially due to an increase in the activity of antioxidant enzymes (superoxide dismutase, peroxidase, and catalase) in the apical part of the main shoot. Moreover, they found that seed priming of berseem clover with salts such as NaCl could also increase seed vigour. Additionally, greater accumulation of Na^+ ions in plants might serve to increase the cell-solute potential and therefore increase their osmotic adjustment (Ambede et al., 2012). The total salt concentration of each water source used in the present study was less than 1100 ppm, which was below the 2000 ppm threshold salt concentration at which salinity has negative effects on seed germination and seedling growth. Hence, some impact on growth was expected from the tubewell water. Thus, the low salinity levels (575±245 ppm) of the irrigation waters used in the present study (Table 1) had no adverse effects on germination, emergence (Table 2) and seedling growth (Table 3).

Quddus et al. (2014) found that small seeds of forage grasses were more susceptible than larger seeds to the negative effects of saline water. However, variation in seed size was unlikely to have been a contributing factor to the significant interaction between water source and seed population, as seeds of uniform size were selected from each seed population in the present study. Salt concentrations below 2000 ppm (as was the case in the present study) have been shown to have a positive effect on EE4DAS due to the effect of increased osmotic potential of saline water on germination, root length and increasing antioxidant enzymes activities (Khatib and Massengale, 1966, Rouhi et al., 2010). Rouhi et al. (2010) also found that osmotic priming resulted in greater energy of emergence. Basra et al. (2005) had also reported similar findings that emergence energy of fine rice was improved with low saline waters due to priming because of efficient mobilization and utilization of seed reserves.

Impact on Seedling Growth and Development

The effects of water source and seed population on seedling growth and development were variable. The significant effects of seed population ($P < 0.05$) on shoot, root and seedling lengths and vigour index are consistent with the studies of Dheeravathu et al. (2021) and Deepak et al. (2010) who used salinity levels with EC values of 4, 8, 12 and 16 dS/m in their studies. The seed population studies of this nature may be important in determining the relationship between root and shoot growth and seedling vigour, and can be used as a tool in the selection process for seed to be grown under the more advantageous saline irrigation water conditions (salty but at “priming levels” – low to moderate levels of salinity) and ultimately for further propagation (Tufail, 2016).

No adverse effects were found in the present study on seed germination and seedling establishment with the use of tube well water. This may be explained by the heavy rains and flooding events which occurred in the area during 2010-11 (prior to this study) which resulting in decreased salt concentration in the subterranean tube well water, as suggested by the differences in tube well water salinity of 2.1 dS/m reported by Mehboob et al. (2011) and 1.6 dS/m recorded in the present study (Table 1). This reduction in salinity made tube well water a suitable alternative source of irrigation for growing the berseem clover crop, especially when canal irrigation water is not available for six months due to the scheduled annual closure of most small canals during the berseem growing winter season from October to March and the two month scheduled closure of the large BSLC canal (from 26th December to 30th January each year) in the study area (Government of Pakistan, 2023).

Independently, water source had no main effect on any of the seedling parameters assessed. However, the results showed that traits of seedling growth and development were positively affected by the moderate salinity levels (1.6 dS/m or 1040 ppm) present in the tube well water (Figure 3), again because of the impact of the 2010 floods on reducing salinity levels in the groundwater. This lack of any adverse effect is most likely due to the relatively low salinity of the irrigation waters used or salinity tolerance shown by the seed populations. However, salts above these concentrations can have detrimental effects on seedling growth and development. These results are in agreement with Deepak et al. (2010) who showed that saline water up to EC of 4-8 dS/m did not affect root and shoot growth and resulted in increased plant biomass production. Similarly, Agarwal and Ahmad (2010) also reported increased shoot and root growth with increases in the concentration of salts in tube well water up to 10 mmol/l concentration with salinity level of <8 dS/m. However, when osmotic concentrations exceeded 50 mmol/l, toxicity occurred resulting in inhibition of seedling emergence, and inhibited seedling growth at salinity level of >8 dS/m and above (Khanduri et al., 2021). Iannucci (2006) also found that shoot elongation was decreased at the early seedling growth and development stage due to the impact of chloride ion interactions on root uptake of ions associated with the use of saline waters.

However, Deepak (2010) found seedling height progressively increased, reaching a maximum height using water with an EC ≤ 8 dS/m, but when this value for EC was exceeded, the seedling height decreased drastically. The positive response in seedling growth may be due to the positive effect of salts on radicle and plumule growth during the first 4 days after sowing (Basra et al., 2005), as found in the present study (Figure 2). Moreover, the small amount of salt in the water increased seedling size and improved seedling fresh weights (Shabbir et al., 2014) observed in sesame seeds, while acting as a priming agent to absorb water for the initiation of primary metabolic activities of the germination process (Rouhi et al., 2010). The lack of effect of water source on fresh weight or dry weight of the shoots and roots is similar to the results of Deepak (2010). Similarly, Ghollarata and Raiesi (2007) also reported no effects of salinity on shoot and root biomass at 2 dS/m.

Impact of Irrigation Water Quality

It appeared that the higher salinity of TW (tubewell) and CTW (50:50 mixture of canal and tubewell) water sources produced a higher seed germination and emergence of Agaitti-Berseem-2002 (AB-2002) than the 2 other seed populations (LBF1 and LBM1). For all growth and development attributes, the 'better quality' water containing lower EC of 0.33 dS/m, SAR of 0.7 mmol/L and a sodium concentration of 0.8 me/L in the canal water treatment (Table 1) performed worst when combined with the market seed (LBM1). When compared with the distilled water (control), no differences in seed germination and emergence were observed between LBF1, LBM1 and AB-2002.

According to the water test results (Table 1), salinity levels in the central Punjab region of Pakistan, particularly from tube wells (1.6 dS/m), were not as severe as previously reported (2.1 dS/m) by Mehboob et al. (2011). In a more recent study carried out in the same region, Tufail et al. (2017) reported that farmers used greater amounts of irrigation water from both canal and tube well sources to grow their berseem clover crops and this could be resulting in enhancing soil properties through leaching of excessive salts and preventing the immediate toxic effects of contaminants. As groundwater quality is influenced by preceding recharge events, it is possible that the flooding that occurred in the region during the 2010 monsoon season (prior to the study taking place) resulted in lowering tubewell salinity levels due to leaching of dissolved salts.

Whilst the irrigation water sources were tested for factors associated with water quality influencing germination and seedling growth, a broader range of tests may have highlighted a difference with the canal water. For example, the presence of elements including sulphur and heavy metals (As, Cd, Co, Cu, Hg, Pb, Ni and Cr) at toxic levels exceeding WHO standards sourced from contaminating sewerage and industrial waste waters (Ashraf et al., 2021), as well as herbicides (Yazdanpak et al., 2014) may have affected both seed germination and seedling development under field irrigation conditions.

CONCLUSION

Using a range of irrigation water sources, water collected in 2014 from within central Punjab (Pakistan), canal water, tube well water or a 50:50 mixture of canal and tube well waters had no major detrimental effects on berseem seed germination or seedling growth. The results further suggested that berseem clover seeds can germinate efficiently (GP of 99.5%) and rapidly (EE4DAS of 77.6%) when irrigated with tube well water with a lower salinity level (up to 1040 ppm or 1.6 dS/m). Further, strong positive correlations were observed among the seedling growth parameters of berseem clover with the use of tube well water. The farmer own-saved seed (LBF1) performed better than the 2 other populations under moderate saline water, and this trait may be valuable for future breeding programs in developing salt-tolerant cultivars of berseem clover. However, further studies are needed to confirm these results at the farm level: broader analysis of water quality factors is also necessary to determine whether salinity, or other contaminants, such as heavy metals, herbicides, and sewerage elements, are influencing results.

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AUTHOR CONTRIBUTIONS

MST conceived and conducted the study, as well as drafted the manuscript. GLK contributed to manuscript editing and project supervision. MSK and AS assisted in refining the manuscript in the context of smallholders' mixed farming systems. JWP and MRN reviewed and assisted in editing and refining the manuscript. PCW secured funding, contributed to manuscript review, and provided overall guidance.

COMPETING OF INTEREST

The authors declare no competing interests.

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