Oregon State University Seed Laboratory

Use of Thermogradient Table in Screening Cover Crops for Cold and Heat Tolerance

Final Report

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EXCUTIVE SUMMARY

Not all cover crops varieties have the same level of cold or heat tolerance. Cover crops with tolerance to cold temperatures would be suitable for planting in the cold winter regions like the Midwest of the US when soil temperature is low in late fall. Whereas varieties with heat tolerance are suitable for the Southern regions of the US. Testing varieties for cold and heat tolerance in the field is costly and time consuming. The objective of this study was to test the validity of the thermogradient table to determine the cold/heat tolerance of annual ryegrass (ARG), radish (RAD), and clover (CLV) varieties.

None of the varieties of the ARG, RAD, and CLV used in the study germinated at 36-40°C range. Varieties within each crop differed significantly in their tolerance to both cold and warm temperatures. The percentage of seed germination, speed of germination index, dry weight content and seedling length tests were suitable for differentiating varieties with different tolerance to cold and warm temperatures. In ARG, variety 2 had the highest tolerance to cold temperatures (10-15°C) with 71% germination and varieties 1 & 3 to warm temperatures (31-35°C) with 46% germination. In RAD, variety 6 had the best tolerance to both cold temperatures (10-15°C) with 75% germination and to warm temperatures (31-35°C) with 68% germination. In CLV, variety 3 had the highest tolerance to both cold (10-15°C) and warm (31-35°C) temperatures with 88% and % 91 germination, respectively. The best germination of all crops was achieved between 16°C and 30°C. The thermogradient table proved to be an effective tool to differentiate between varieties with different tolerance levels to cold and warm temperatures in ARG, RAD, and CLV. Screening for cold/heat tolerances using the thermogradient germination test can help growers and seed companies to make quick decisions on selecting the best varieties for cold or warm-regions to warrant a successful stand establishment. It is a viable alternative to the field trials for screening varieties for cold and heat tolerance that saves time and money for farmers and seed industry.

Objective

Screen different varieties of annual ryegrass, clover, and radish for cold and heat tolerance using a thermogradient table (TGT).

Justification

Tolerances of crop varieties to cold or heat is controlled by genetic factors. For example, the soybean varieties planted in Alberta, Canada (53.9333° N, 116.5765° W) are adapted to the cold climate of the region, whereas soybean varieties grown in Mexico (23.6345° N, 102.5528° W) are adapted to the warm climate ~~in that region~~. Not all varieties of the same crop have the same level of cold or heat tolerance. Cover crops with tolerance to cold temperatures would be suitable for planting in the Midwest region of the US in fall when soil temperatures are as low as 7-10℃, Varieties with cold tolerance have high probability of fast field emergence to survive the cold winter. Varieties with tolerance to warm temperatures would be suitable for planting in the Southern part of the US. The use of TGT was reported to be a valuable tool for screening different varieties of various crops for cold and heat tolerance (Welbaum et al., 2016). Yet, it has not been used in seed testing community to help the seed industry select and planting the proper varieties that are best fitting a particular geographical region. This study intended to validate the usefulness and practicality of using TGT in identifying varieties with cold and heat tolerance. The application will benefit both seed industry and seed testing laboratories. This study recommended steps to conduct TGT germination test and identified observation and parameters to collect. Interpreting and reporting the results to customers were also discussed.

Background

The TGT can be used for many applications, including a) screening crop varieties for cold and heat tolerance, b) identifying the optimum range of temperatures for germination of various crops, and c) determining the quality of different seed lots, higher quality seeds germinate in a wider temperature range than lower quality seeds. Screening varieties for cold and heat tolerance by TGT takes considerably shorter time compared to the costly, lengthy field trials. It is innovative, efficient technology for farmers interested in selecting and planting proper cover crop varieties in particular environmental conditions to increase the probability of good stand establishment.

Cover crops such as annual ryegrass (ARG) *Lolium multiflorum*, radish (RAD) *Raphanus sativus* and clover (CLV) *Trifolium pretense, T. repens, T. incarnatum*, are planted in different regions of the United States and other areas of the world to reduce soil erosion, restore organic matter, improve soil quality, and retain nutrients and moisture (Bacq-Labreuil et al., 2019). The ARG has the advantage of growing in poorly drained soil in no-till farming systems (Hart et al., 2011) and is easy to kill in the spring after it fulfills its function as a cover crop in the fall and winter (Plumer et al., 2016). Radish has good biomass, which adds to the soil organic matter, and has sizable root system that improves soil structure. Clover has the advantage of fixing nitrogen and cutting fertilizer cost.

Two factors are considered when screening for cold tolerance of cover crops. The first is the speed of germination, i.e., how fast the seeds germinate under cold temperatures (~10°C) before colder weather arrives in late fall in the Midwest region. The second is seedling size before temperatures drop to the freezing point. Reasonable seedling size (~5–6 inches) is needed for the plant to survive the cold winter. Smaller seedlings are more susceptible to winter kill. The same can be said for screening for heat-tolerance with higher temperature of ~30-35°C (Elias et al., 2017). Both factors can be achieved using the TGT by recording germination and measuring seedling length and dry weight. The alternative method for screening varieties for cold/heat tolerance is to conduct research studies in the field, which is laborious, costly and time consuming. In addition, when screening varieties under unpredicted field conditions, many factors such as drought, excessive rainfall, or extreme temperatures, might interfere with the study and complicated the screening process.

One of the main challenges of using cover crops in the Midwest is selecting varieties that will tolerate very low temperatures (~ 5–10°C/41–50°F) and produce good stand establishment. Although ARG is a cool-season grass, some varieties have better cold tolerance than others, depending on the genetic makeup of each variety. Asomaning et al. (2011) reported that using a TGT is an effective method for predicting seed germination of *Terminalia superba* under cold climatic conditions (5–15°C/41–59°F).

Although planting in September is suggested in the Northern Corn Belt and by mid-October in the Southern Corn Belt, above normal rainfall in some years may delay planting. In such cases, seeds may be planted late, and would germinate under suboptimal soil temperatures. Thus, selecting varieties that tolerate cold is critical in order to have a better chance of quick, uniform emergence. This research was designed to use thermogradient table to screen several varieties of ARG, radish, and clover for cold and heat tolerance. Eight ARG, six RAD, and six CLV varieties were planted and studied under six temperature ranges (TR) of 10-15°C, 16-20°C, 21-25°C, 26-30°C, 31-35°C, and 36-40°C on a thermogradient table (TGT).

MATERIALS AND METHODS

Eight annual ryegrass (ARG), six radish (RAD), and six clover (CLV) varieties were used in the study (Table 1). They were screened for cold and heat tolerance under six temperature ranges (TR). The temperature ranges at which seeds were planted are 10–15°C, 16–20°C, 21–25°C, 26–30°C, 31–35°C, and 36–40°C. Tetrazolium and standard germination tests were conducted according to the 2023 AOSA Rules for Testing Seeds and the Tetrazolium Handbook to determine the initial viability of varieties used in the study.

Table 1. List of Annual ryegrass (ARG), radish (RAD), and clover varieties used in the study.

Crop	Variety	Production year
Lolium multiflorum (ARG)	1.Ca	2022
	2.As	2023
	3.Ra	2022
	4.DT	2023
	5.At	2022
	6.BB	2023
	7.Di	2022
	8.Fi	2022
Raphanus sativus (RAD)	1.Ni	2023
	2.Ta	2023
	3.Ar	2023
	4.CG	2022
	5.Ae	2022
	6.FB	2023
Trifolium pratense (Red clover)	1.Bl	2023
	2.Me	2023
Trifolium	3.Fl	2023
Trifolium incarnatum (Crimson clover)	4.N/S	2023
	5.Co	2022
	6.Al	2022

High quality seeds were used in the study to exclude the possibility that results observed were due to seed quality. The cold/heat stress is the main factor that affects the screening process.

- Germination was determined to be realized and recorded when root and shoot emergence reached 0.5 cm or longer.
- Number of germinating seeds in each temperature range were counted and reported on day 7.
- Average seedling linear length (SL) were measured and recorded (cm) from the top of the leaves to the end tip of the roots.

- Five randomly selected seedlings were chosen from each replication of each treatment and dried in oven for 24 hours at 100°C. Dry weight (DW) was recorded (mg/seedling) at the end of drying period.
- Speed of germination indices (SGI) were calculated at the end of the final counts. The higher the SGI, the faster the germination. In addition, varieties with higher SGI at low temperature range indicates better emergence success rate in the Midwest environment. Varieties with higher SGI at high temperature range indicates better potential emergence success in warm regions of the US, such as Alabama, Louisiana, and Mississippi. The speed of germination test was recorded at day 7 to reflect how fast seeds of different varieties germinated. More seedlings may germinate over longer period of time, especially in colder temperature.
- The dimensions of the thermogradient table (TGT) are 60 inches (152 cm) length x 40 inches (102 cm) width x 9 inches (23 cm) depth (Fig. 1). Materials used in growing seeds in the TGT were: 1) brown germination paper, 2) white germination paper and 3) plastic grids. One seed was planted in each cell (Fig. 1).

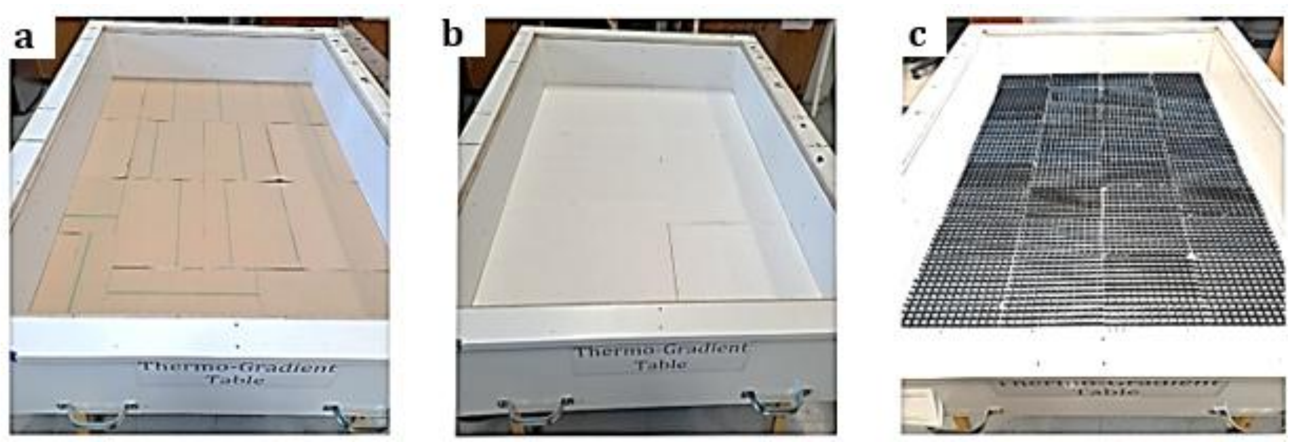

Figure 1. Thermogradient table with a) brown germination paper on the bottom, b) white germination paper on top of the brown paper, and c) plastic divider grids on the white germination paper. One seed was planted in each cell to ensure accurate evaluation.

- For each seed lot and each temperature range, two replications of one-hundred seeds each were tested. Seeds were divided into six temperature ranges. Temperature ranges were measured using a NIST certified digital thermometer and laser gun thermometer. The hydration of the substrate was checked twice daily. The counts of germinated seeds were taken on day 7. The linear length and the dry weights of five randomly selected seedlings from each replicate of each treatment (temperature ranges, and varieties) were measured and recorded.

Statistical Analysis

ANOVA, LSD test at $p \leq 0.05$, means, maximum, and minimum values were used to analyze the data and rank varieties for cold and heat tolerance. Four replications were used in the standard germination tests and two replications in the TZ tests. The number of seeds in each temperature range were counted separately. Each crop was analyzed separately because the focus of the study was not to compare crops.

RESULTES AND DISCUSSION

Initial viability of seed samples used in the study

Table 2 shows the initial viability by the TZ and the standard germination test results of ARG, RAD, and CLV varieties used in the study. TZ results ranged between 92% and 99%, and germination results between 89% and 99% for all crops and varieties. Seeds were considered germinated when radicle and cotyledon reached a minimum of 0.5 cm in length. High quality seeds were used in the study to make sure that the results observed were due to the effect of temperature ranges not due to the quality of the seeds.

Table 2. Seed viability by tetrazolium and standard germination tests for the crops and varieties used in the study.

Species	Variety	Year	Viability by TZ (%)	Germination (%)
Lolium multiflorum (ARG)	1. Ca	2022	96	97
	2. As	2023	97	98
	3. Ra	2022	92	98
	4. DT	2023	97	98
	5. At	2022	97	98
	6. BB	2023	98	97
	7. Di	2022	97	97
	8. Fi	2022	97	98
Raphanus sativus (RDH)	1. Ni	2023	99	99
	2. Ta	2023	99	99

	3. Ar	2023	98	97
	4. CG	2022	99	98
	5. Ae	2022	99	99
	6. FB	2023	99	99
Trifolium pratense (Red clover)	1. Bl	2023	97	95
	2. Me	2023	96	94
	3. Fl	2023	94	92
Trifolium incarnatum (Crimson clover)	4. N/S	2023	98	92
	5. Co	2022	94	89
Trifolium repens (White clover)	6. Al	2022	97	94

Effect of temperature on germination, SGI, DW, and SL of different annual ryegrass, radish, and clover varieties

The ANOVA results showed that varieties, temperature, and their interactions had significant effect on germination, SGI, SL, and DW. The number of seedlings that germinated at each temperature range (10–15°C, 16–20°C, 21–25°C, 26–30°C, 31–35°C, and 36–40°C) and among varieties differed significantly. The same trend was found in seedling length and dry weight content (Table 3).

The interactions between varieties and temperatures ranges were significant with only one exception, in DW of ARG. This indicates that varieties behaved differently in response to the temperature ranges in ARG, RAD, and CLV (Table 3).

Table 3. Analysis of variance for the effect of six temperature ranges on germination, speed of germination index (SGI), seedling length (SL), and dry weight content (DW) of eight annual ryegrass varieties, six radish, and six clover varieties.

Crop	Source of variation	df	Germination	SGI	Seedling length (SL)	Dry weight content (DW)
			Probability (0.05)			
ARG	Varieties (V)	7	***	***	***	*
	Temperature Range (T)	5	***	***	***	***
	(V) x (T)	35	*	**	***	ns
RDH	Varieties (V)	5	***	***	***	***
	Temperature Range (T)	5	***	***	***	***
	(V) x (T)	25	***	***	***	***
CLV	Varieties (V)	5	***	***	***	**
	Temperature Range (T)	5	***	***	***	***
	(V) x (T)	25	***	***	**	***

*** highly significant at $P \leq 0.001$, ** significant at $P \leq 0.01$, * significant at $P \leq 0.05$, and ns, not significant.

I. Annual Ryegrass

a) Germination of ARG varieties at different temperature ranges

Varieties differed in tolerance to different temperature ranges (Fig. 2). None of the varieties germinated at 36-40°C. Varieties 2, 1, and 5 had the highest germination at 10-15°C after 7 days with 71%, 67%, and 67%, respectively (Fig. 2). These varieties can be good candidates to grow as cover crops in the Midwest, and low temperature regions. The highest germination was achieved at 21-25°C, followed by 16-20°C, and 26-30°C, at average of 96%, 95%, and 91% over the eight varieties, respectively (data not shown). Varieties 1, 3, and 6 had the best germination at 31-35°C with 46%, 46%, and 42%, respectively. These results suggest that certain varieties are better suited for specific temperature conditions.

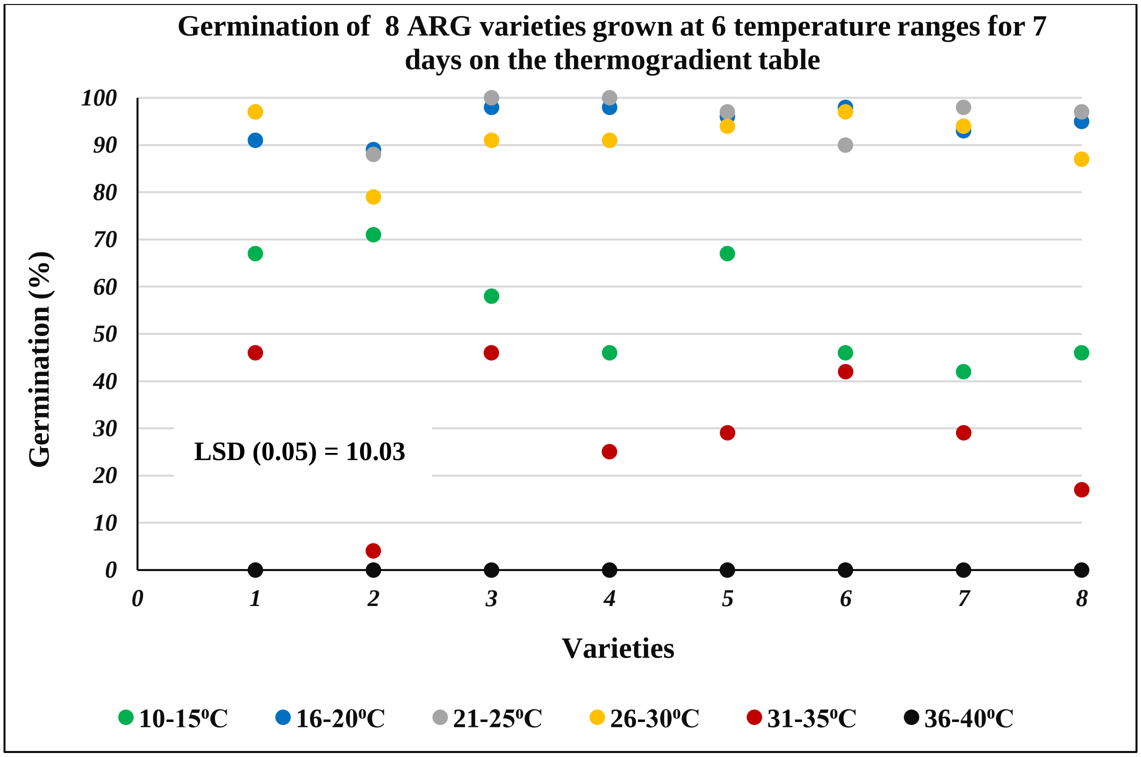

Figure 2. Germination of eight ARG varieties grown at six temperature ranges for 7 days on the thermogradient table.

b) Speed of Germination Index (SGI)

None of the varieties germinated at 36-40°C. Varieties 2, 5, and 1 had the highest SGI at 10-15°C with 2.1, 2.0, and 1.9, respectively. These varieties can be good candidates for cover crops in the Midwest, and other low temperature regions. However, the speed of germination at 10-15°C was generally low (Table 4). The highest SGI was achieved at 26-30°C followed by 21-25°C, and 16-20°C (Table 4), indicating that faster growth rate happened at higher temperature ranges. At 31-35°C, the SGI was low for all varieties as the seedling growth slowed considerably. It is worthy to note that some varieties start emerging slow but catch up later. Therefore, SGI should not be taken as the sole vigor index.

Table 4. Speed of germination index (SGI) and average seedling of eight ARG varieties grown at six temperature ranges on a thermogradient table.

	Speed of Germination Index (SGI)								
Temp Ranges	***1. Ca***	***2. As***	***3. Ra***	***4. DT***	***5. At***	***6. BB***	***7. Di***	***8. Fr***	***Mean***
10-15°C	1.9	2.1	1.8	1.6	2.0	1.6	1.6	1.7	1.8
16-20°C	5.6	5.4	6.0	5.9	5.9	6.0	5.7	5.8	5.8

21-25°C	6.3	5.8	6.4	6.4	6.3	6.0	6.4	6.3	6.2
26-30°C	7.1	5.9	6.8	6.8	6.9	7.1	7.0	6.4	6.8
31-35°C	1.3	0.3	1.4	0.95	0.87	1.1	0.97	0.69	0.9
36-40°C	***None of the seedings germinated (0)***								
Mean	***3.9***	***3.6***	***4.2***	***4.3***	***4.5***	***4.6***	***4.8***	***4.8***	
LSD$_{(0.05)}$	***0.68***								

c) **Dry weight content (DW) of eight ARG varieties grown at different temperature ranges**

None of the varieties germinated at 36-40°C. The average of the highest dry weight over the 8 varieties was achieved in temperature ranges of 21-25°C, 26-30°C, and 31-35°C, with 10.0, 10.3, and 10.6 mg/seedling, respectively; followed by 16-20°C, and 10-15°C, with 9.7 and 8.9 mg/seedling, respectively. This indicated that at warmer temperatures, the dry weight content increased. These results are consistent with the SGI results. Varieties No. 1, 4, and 5 showed the highest average dry weight content over all temperature ranges, with 12.12, 11.04, and 11.0 mg/seedling, respectively, whereas varieties 8 and 3 had the lowest dry weight content, with 7.48 and 8.12mg/seedling, respectively (Table 5).

Table 5. Dry weight content of eight ARG varieties grown at six different temperature ranges on a thermogradient table.

Temp Range	**Varieties**								**Mean**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
	Dry weight (mg/seedling)								
10-15°C	11.1	7.9	7.2	10.1	10.6	10.0	8.7	5.6	***8.9***
16-20°C	12.0	9.3	7.9	10.8	11.1	10.4	9.0	7.0	***9.7***
21-25°C	12.4	9.9	8.2	11.0	11.1	10.7	9.2	7.8	***10.0***
26-30°C	12.7	10.4	8.5	11.3	11.2	10.9	9.4	8.3	***10.3***
31-35°C	12.4	10.5	8.8	12.0	11.0	11.4	9.6	8.7	***10.6***
Mean	***12.12***	***9.60***	***8.12***	***11.04***	***11.00***	***10.68***	***9.18***	***7.48***	
36-40°C	***None of the seedings germinated (0)***								
LSD $_{(0.05)}$	**2.35**								

Max	***12.7***	***10.5***	***8.8***	***12.0***	***11.2***	***11.4***	***9.6***	***8.7***	
Min	***11.1***	***7.9***	***7.2***	***10.1***	***10.6***	***10.0***	***8.7***	***5.6***	

d) Seedling length (SL) of eight ARG varieties grown at different temperature ranges

None of the varieties germinated at 36-40°C. The highest seedling length was achieved at temperature ranges of 21-25°C, 16-20°C, and 26-30°C with an average, over the eight varieties, of 19.8, 17.8, and 17.3 cm, respectively. These results are consistent with SGI and dry weight contents. Average short seedling length of 11.7 cm was recorded at 10-15°C, and the shortest seedling length of 2.7 cm was recorded at 31-35°C, indicating that extreme cold or warm temperatures affect the growth rate of seedlings. Varieties 1 and 4 showed the highest seedling length, with an average of 13.76 and 13.12 cm, respectively, followed by varieties 7 and 6, with 12.30 and 12.15 cm, respectively. Varieties 8 and 2 had the lowest seeding length with 8.4 and 10.14 cm, respectively (Table 6).

Table 6. Seedling length of eight ARG varieties grown at six different temperature ranges on a thermogradient table.

Temp Range	**Varieties**								**Mean**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	
	Seedling length (cm)								
10-15°C	12.98	8.84	11.06	13.01	13.32	12.74	11.54	10.01	***11.7***
16-20°C	22.30	16.40	14.73	20.45	17.04	19.64	18.19	13.87	***17.8***
21-25°C	24.29	15.53	20.59	19.48	20.13	20.96	22.55	14.82	***19.8***
26-30°C	19.60	16.09	18.52	22.18	15.73	17.39	18.39	10.15	***17.3***
31-35°C	3.38	3.02	2.77	3.63	2.25	2.19	3.11	1.58	***2.7***
Mean	***13.76***	***10.14***	***11.28***	***13.12***	***11.41***	***12.15***	***12.30***	***8.40***	
36-40°C	***None of the seedings germinated (0)***								
Max	***24.29***	***16.40***	***22.59***	***22.18***	***20.13***	***20.96***	***22.55***	***14.82***	
Min	***3.38***	***3.02***	***2.77***	***3.63***	***2.25***	***2.19***	***3.11***	***1.58***	
LSD $_{(0.05)}$	***1.02***								

II. Radish

a) Germination of radish (RAD) varieties at different temperature ranges

Radish varieties differed in their tolerance to different temperature ranges (Fig. 3). None of the varieties germinated at 36°C-40°C. Varieties 6, and 4 had the highest germination at 10-15°C after 7 days with 75% and 65%, respectively (Fig. 3), indicating that these varieties can be good candidates for cover crops in the Midwest, and other low temperature regions. The highest germination was achieved at 21-25°C, followed by 26-30°C, and 16-20°C, at average of 92%, 92%, and 86% over the six varieties, respectively (Fig. 3). These results suggest that radish seeds germinate at temperature range of 16°C-30°C better than lower than 16°C or higher than 30°C.

Variety 6 had the best germination at both the lowest (10-15°C) and the highest (31-35°C) temperature ranges, as well as at all temperature ranges with an average of 86%, compared to an average of 68.5% for the other 5 varieties (Fig. 3).

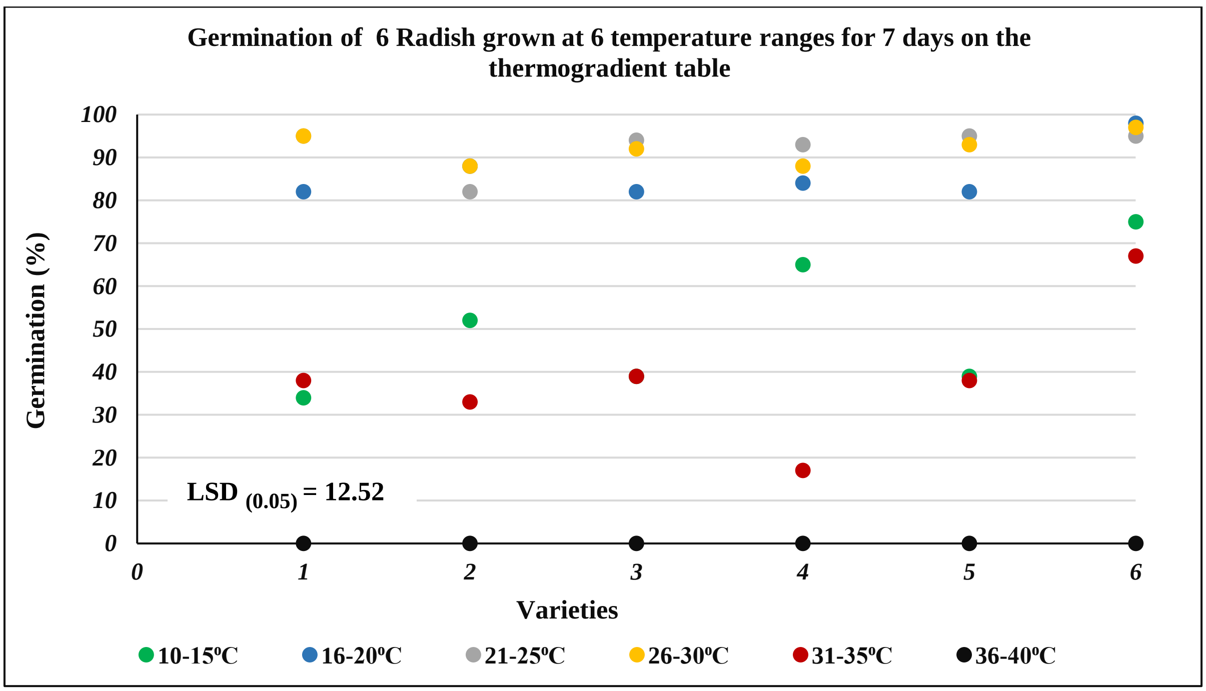

Figure 3. Germination of six radish varieties grown at six temperature ranges for 7 days on the thermogradient table.

b) Speed of germination index (SGI) of radish

None of the varieties germinated at 36-40°C. Variety 4 had the highest SGI at the temperature range of 10-15°C with 22.0 followed by variety 6 with 18.4, suggesting that they performed the best in low temperatures compared to the other varieties, and they are suitable for planting in low-temperature regions. Varieties 1 and 5 were extremely slow with 7.3 and 9.9, respectively.

The highest average SGI over all temperature ranges was 18.6 achieved in varieties 6 followed by varieties 3 and 4 with 18.2 and 16.2, respectively (Table 7). Variety 3 had the highest SGI in the temperature range of 26-30°C, with 23.3, followed by variety 6 with 16.6. Varieties 1 and 2 had the lowest average SGI over all temperature ranges with 13.8 and 14.6, respectively (Table 7).

These results are similar to SGI of ARG, indicating that faster germination of radish seeds occurs between 16°C and 30°C.

Table 7. Speed of germination index (SGI) and average seedling of six radish (RAD) varieties grown at six temperature ranges on a thermogradient table.

Temp	**Speed of Germination Index of Radish (RAD)**						
Range	*1. Ni*	*2. Ta*	*3. Ar*	*4. CG*	*5. Ae*	*6. FB*	***Mean***
10-15 °C	7.3	13.3	12.9	22.0	9.9	18.4	***13.9***
16-20°C	19.8	21.6	23.0	21.8	20.5	23.9	***21.8***
21-25°C	23.4	20.2	23.4	23.0	23.6	23.4	***22.8***
26-30 °C	23.3	21.9	23.8	22.1	22.7	23.5	***22.9***
31-35 °C	8.1	8.9	23.3	4.1	10.8	16.6	***12.6***
36-40 °C	***None of the seedlings germinated (0)***						
Mean	***13.8***	***14.6***	***18.2***	***16.2***	***15.4***	***18.6***	
LSD $_{(0.05)}$	***7.1***						

c) Dry weight content (DW) of radish (RAD) varieties at different temperature ranges

None of the varieties germinated at 36-40°C. The highest average dry weight overall varieties was achieved in temperature ranges of 21-25°C and 26-30°C, with 50.84 and 50.21 mg/seedling, respectively, followed by 44.80 mg/seedling at 16-20°C (Table 8). Temperature ranges of 31-35°C and 10-15°C, had the least dry weight contents with 35.77 and 35.35 mg/seedling, respectively. This is a similar trend to ARG where the highest dry wight content was achieved between 16°C and 30°C.

This indicates that at warmer temperatures, the dry weight content increased. However, at extreme high temperatures of 31-35°C or low temperature of 10-15°C, the dry weight content was the lowest.

Varieties 1 and 3 showed the highest overall dry weight with 51.48 and 46.27 mg/seedling, respectively. Varieties 4, 5 and 6 had lower dry weight content of 42.52, 40.98 and 40.24 mg/seedling, respectively. Variety 2 had the lowest dry weight, with an average of 38.89 mg/seedling (Table 8). Variety 4 had the highest dry weight content at temperature range of 10-15°C at 39.19. Seedlings must be big in size in order to withstand the extreme cold temperatures in winter (Elias et al. 2017).

Table 8. Dry weight content of six radish varieties grown at 6 different temperature ranges on a thermogradient table.

Temp Range	**Varieties**						**Mean**
	1	**2**	**3**	**4**	**5**	**6**	
	Dry weight (mg/seedling)						
10-15°C	36.26	33.69	36.10	39.19	34.07	32.79	***35.35***
16-20°C	51.30	38.23	48.28	47.93	46.03	37.05	***44.80***
21-25°C	58.15	41.68	57.55	54.98	49.95	42.73	***50.84***
26-30°C	68.58	46.68	51.53	39.45	45.48	49.55	***50.21***
31-35°C	43.11	34.15	37.88	31.04	29.35	39.09	***35.77***
Mean	***51.48***	***38.89***	***46.27***	***42.52***	***40.98***	***40.24***	
36-40°C	***None of the seedings germinated (0)***						
LSD $_{(0.05)}$	***6.8***						
Max	***16.49***	***13.99***	***15.79***	***15.12***	***21.95***	***17.6***	
Min	***4.78***	***4.69***	***4.23***	***5.09***	***5.72***	***5.94***	

d) Seedling length (SL) of six radish varieties grown at different temperature ranges

None of the varieties germinated at 36-40°C. The highest seedling length was achieved at temperature ranges of 21-25°C, 26-30°C, and 16-20°C with an average of overall the six varieties of 16.82 cm, 13.27 cm, and 11.15 cm, respectively. The lowest seedling length was recorded at 10-15°C with 5.08 cm, followed by 7.94 cm at 31-35°C.

These results confirmed the ARG findings that the highest growth rate expressed by seedling length and dry weight was achieved between 16°C and 30°C, but it slows down at 10-15°C, and 31-35°C.

Varieties 5, 6, and 1 showed the highest seedling length over all temperature ranges, with an average of 14.15, 11.34, and 11.06 cm, respectively, followed by varieties 4, 2, and 3, with an average of 9.96, 9.40, and 9.20 cm, respectively (Table 9).

Table 9. Seedling length of six radish varieties grown at 6 different temperature ranges on a thermogradient table.

Temp Range	**Varieties**						**Mean**
	1	**2**	**3**	**4**	**5**	**6**	
	Seedling length (cm)						
10-15°C	4.78	4.69	4.23	5.09	5.72	5.94	5.08
16-20°C	13.60	8.73	9.61	11.04	12.61	11.33	11.15
21-25°C	16.49	13.99	15.79	15.12	21.95	17.60	16.82
26-30°C	11.13	13.08	11.83	10.50	20.42	12.68	13.27
31-35°C	9.31	6.52	4.54	8.07	10.04	9.17	7.94
36-40°C	***None of the seedings germinated (0)***						
LSD $_{(0.05)}$	**2.3**						
Mean	***11.06***	***9.40***	***9.20***	***9.96***	***14.15***	***11.34***	
Max	***16.49***	***13.99***	***15.79***	***15.12***	***21.95***	***17.6***	
Min	***4.78***	***4.69***	***4.23***	***5.09***	***5.72***	***5.94***	

III. Clover

a) Germination of clover (CLV) varieties at different temperature ranges

Clover varieties differed in their tolerance to different temperature ranges (Fig. 4). None of the varieties germinated at 36-40°C. After 7 days, variety 3 had the highest germination of 88% at the temperature range of 10-15°C followed by variety 5 at 81%, indicating that those varieties have potential to germinate well in low temperature regions (Fig. 4). The highest average germination over the six varieties was achieved at 21-25°C with 92% , and the lowest with 69% at the temperature range of 31-35°C. The average germination of other temperatures ranged between 73% and 85% (data not shown). These results suggested that the best germination rate of clover seeds was between 16-30°C.

Variety 3 had the best germination at both the lowest temperature (10-15°C) with 88% and the highest temperature (31-35°C) with 97%, as well as the highest average germination over all temperature ranges with 91%, followed by variety 5 with 85%, and variety 6 with 83%. The average germination of the other varieties over all temperature ranges was 73-80%. Variety 2 had the lowest germination at 10-15°C range and variety 4 and the lowest germination at 31-35°C range (Fig. 4).

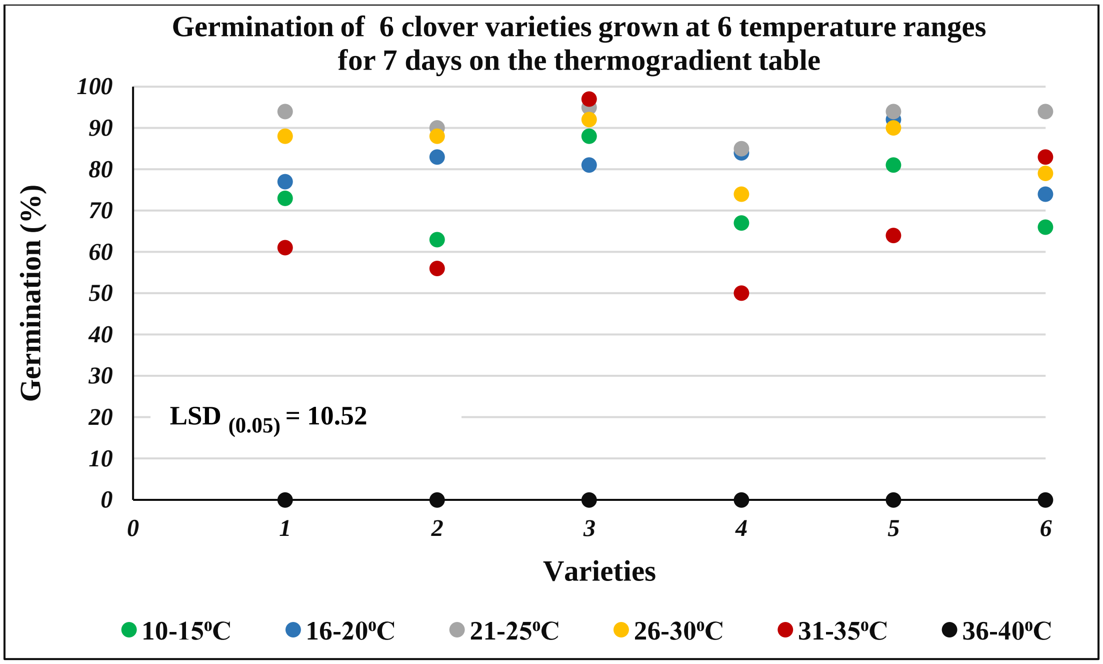

Figure 4. Germination of six clover varieties grown at six temperature ranges for 7 days on the thermogradient table.

b) Speed of germination index of clover

None of the varieties germinated at 36-40°C. Variety 3 had the highest SGI at the temperature range of 10-15°C with 20.8 followed by variety 5 with 19.5, suggesting that they performed the best in low temperatures compared to the other varieties, and they are suitable for planting in low-temperature regions. Varieties 2 and 6 were extremely slow with 15.1 and 15.9, respectively (Table 10).

The highest average SGI over all temperature ranges was 18.7 achieved in varieties 3 followed by varieties 5 and 6 with 17.6 and 16.8, respectively (Table 10). Variety 3 had the highest SGI in the temperature range of 26-30°C, with 23.3, followed by variety 6 with 19.9. Variety 4 had the lowest average SGI over all temperature ranges with 15.0 (Table 10).

Table 10. Speed of germination index (SGI) and average seedling of six CLV varieties grown at six temperature ranges on a thermogradient table.

Temp Range	**Speed of Germination Index of Clover (CLV)**						
	1. Bl	*2. Me*	*3. Fl*	*4. N/S*	*5. Co*	*6. Al*	***Mean***
10-15 °C	17.6	15.1	20.8	16.3	19.5	15.9	***17.4***
16-20°C	18.4	20.1	19.5	20.3	22.1	17.8	***19.7***
21-25°C	22.9	21.9	23.1	20.5	22.6	22.7	***22.3***
26-30 °C	20.9	21.5	22.3	17.8	21.5	18.8	***20.5***
31-35 °C	13.3	12.8	23.3	11.2	15.1	19.9	***15.9***
36-40 °C	***None of the seedlings germinated (0)***						
Mean	***15.7***	***15.6***	***18.7***	***15.0***	***17.6***	***16.8***	
LSD $_{(0.05)}$			***5.07***				

c) Dry weight content (DW) of clover (CLV) varieties at different temperature ranges

None of the varieties germinated at 36-40°C. Varieties 4 and 1 had the highest dry weight content at temperature ranges of 10-15°C, with 1.57 and 1.54 mg/seedling, respectively (Table 11), suggesting that these varieties had good potential to grow at regions of low temperatures. Varieties 4 and 1 had the highest dry weight of all temperature ranges. Variety 2 had the lowest dry weight contents at all temperature ranges, with average of 0.32 mg/seeds. Varieties, 3, 5, and 6 had dry weigh content between 0.65 and 0.77 mg/seedling.

The dry weight contents at warmer temperatures of 21-25°C and 26-30°C increased compared to temperature ranges of 10-15°C and 16-20°C. These results suggested that some varieties, such as 4 and 1, have high quality seeds and germinate well at both low and high temperature ranges.

Table 11. Dry weight content of six clover varieties grown at 6 different temperature ranges on a thermogradient table.

Temp Range	**Varieties**						**Mean**
	1	**2**	**3**	**4**	**5**	**6**	
	Dry weight (mg/seedling)						
10-15°C	1.54	0.30	0.62	1.57	0.61	0.66	***0.88***
16-20°C	1.29	0.29	0.76	1.61	0.60	0.55	***0.85***
21-25°C	1.83	0.30	1.11	1.79	0.68	0.60	***1.05***
26-30°C	1.67	0.44	0.76	1.77	1.06	0.62	***1.05***
31-35°C	1.96	0.29	0.61	1.59	0.71	0.83	***1.00***
36-40°C	***None of the seedings germinated (0)***						
LSD $_{(0.05)}$	**0.12**						
Mean	***1.66***	***0.32***	***0.77***	***1.67***	***0.73***	***0.65***	
Max	***1.96***	***0.4375***	***1.105***	***1.785***	***1.055***	***0.83***	
Min	***1.29***	***0.29***	***0.61***	***1.57***	***0.60***	***0.55***	

d) Seedling length (SL) of six clover varieties grown at different temperature ranges

None of the varieties germinated at 36-40°C. Varieties 4 and 1 had the highest seedling length at temperature ranges of 10-15°C, with 6.74 and 6.32 cm, respectively (Table 12), suggesting that these varieties had good potential to grow at regions of low temperatures. These results are similar to the dry weight content results indicating that these varieties had highest growth rate at low temperature range of 10-15°C.

The highest seedling length was achieved at temperature ranges of 21-25°C, 16-20°C, and 26-30°C with an average of overall the six varieties of 7.15, 6.91, and 6.65 cm, respectively. The lowest seedling length was recorded at 10-15°C with 4.91 cm, followed by 5.41 cm at 31-35°C (Table 12). These results confirmed the ARG findings that the highest growth rate expressed by seedling length and dry weight was achieved between 16°C and 30°C, but slowed down at 10-15°C, and 31-35°C.

Table 12. Seedling length of six clover varieties grown at 6 different temperature ranges on a thermogradient table.

Temp Range	**Varieties**						***Mean***
	1	**2**	**3**	**4**	**5**	**6**	
	Seedling length (cm)						
10-15°C	6.32	2.97	4.23	6.74	5.01	4.19	***4.91***
16-20°C	12.19	3.28	5.72	8.81	5.31	6.13	***6.91***
21-25°C	9.66	4.02	6.72	9.73	6.57	6.19	***7.15***
26-30°C	9.47	3.96	6.04	8.87	5.54	6.04	***6.65***
31-35°C	7.36	4.09	4.28	6.47	5.14	5.14	***5.41***
36-40°C	***None of the seedings germinated (0)***						
LSD $_{(0.05)}$	**0.78**						
Mean	***9.00***	***3.66***	***5.40***	***8.12***	***5.51***	***5.54***	
Max	***12.19***	***4.09***	***6.72***	***9.73***	***6.57***	***6.19***	
Min	***6.32***	***4.97***	***4.23***	***6.47***	***5.01***	***4.19***	

Varieties 1 and 4 showed the highest seedling length, with an average of 9.00 and 8.12 cm, respectively overall the temperature ranges, followed by varieties 6, 5, 3, and 2, with an average of 5.54, 5.51, 5.40, and 3.66 cm, respectively (Table 12).

CONCLUSIONS

- None of the varieties of the annual ryegrass, radish, and clover tested germinated at 36°C-40°C range.
- The varieties of the three crops used in the study differed significantly in their tolerance to both cold and warm temperatures.
- Germination rate, speed of germination index, dry weight content, and seedling length were effective parameters to measure the tolerance of different varieties of ARG, RAD, and CLV to cold and warm temperatures.

- In annual ryegrass, variety 2 had the highest tolerance to cold temperatures (10-15°C) with 71% germination and varieties 1 & 3, to warm temperatures (31-35°C) with 46% germination.
- In radish, variety 6 had the highest tolerance to cold temperatures (10-15°C) with 75% germination and to warm temperatures (31-35°C) with 68% germination.
- In clover, variety 3 had the highest tolerance to cold temperatures (10-15°C) with 88% germination and to warm temperatures (31-35°C) with % 91 germination.
- The best germination of all varieties and crops was achieved between 16°C and 30°C.
- Thermogradient table proved to be an effective tool to differentiate tolerance to cold and warm temperatures among varieties in annual ryegrass, radish, and clover.
- For future studies, it is recommended to conduct screening for tolerances to cold and to warm tolerance temperatures in separate tests. The TGT runs more efficiently when the gap between extreme temperatures does not stretch too wide (i.e., very low, 5°C and very high, 40°C).
- Thermogradient tables can be used by seed labs to help growers and seed companies to make quick decisions on selecting the best varieties to suit local needs that warrants a successful stand establishment of cover crop. It is a viable alternative to the field trials for screening varieties for cold and heat tolerance, which saves time and money.

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