

# Maize (*Zea mays L*) seed germination and seedling growth under varying hydrothermal conditions

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**Abstract** - Varying hydrothermal conditions i.e. changes in water potential and temperature affect maize seed germination and seedling growth. Major effects of climate change are increase in daily temperatures and decrease in rainfall which in turn result in low available soil moisture. From experiments carried out (three maize cultivars seed germination test at five water potentials and five temperatures, three maize cultivars seed hydro-priming at three priming periods i.e. 12h, 18h and 24h, and lastly seedling growth experiment with three maize cultivars at the same temperature of 25°C with five water potentials. Experiments for the three objectives were conducted at University of Pretoria at the main Campus in Hatfield South Africa. The main objective of the study was to determine differences among maize cultivars in germination and seedling growth under varying hydro-thermal conditions in South Africa. At low temperature of 15°C, seed germination was below 60% for the three cultivars. This is supported by previous researches that established that base temperature for maize seed germination is +/- 10°C. Optimum temperature and optimum water potential whereby majority of the maize seed germinated were 20-30°C and -0.1 to 0 MPa for all the three maize cultivars whereby 97.5% germination was realised. Optimum hydro-priming period was realised to be 18h for it was after these hours that all the three cultivars had highest germination percentage,  $T_{50}$  and germination index. The third experiment was on maize seed cultivars emergence and seedling growth. The experiment was a complete random block design replicated three times. Optimum seedling emergence, seedling growth and stomata conductance was observed on seeds hydro-primed for 18h and having available moisture of 50-55%. Factors like cultivar, available soil moisture and hydro-priming period had significant effect on final emergence period, seedling growth and stomata conductance. PAN4A-111 had the highest results in terms of final emergence percentage, growth traits and stomata conductance. Since the whole study was looking at the effect of hydrothermal conditions on germination and seedling growth of maize, it is best for farmers to choose PAN4A-111. In conclusion based on the three experiments in this study, used to test the hypotheses that varying hydrothermal conditions i.e. temperature and water potential have significant effect on maize seed germination and seedling growth the experiment results proved this to be correct. Low temperature and supra-optimal temperature (above 30°C) result in reduced final germination percentage. Low water potential from -0.4MPa at all temperatures result in low daily germination percentage, increased  $T_{50}$ , low final germination percentage and reduced germination rate index. Farmers have to plant when their water potential is near neutral i.e. -0.1 to 0 MPa. The study was handy in

**Index Terms**- Hydrothermal, water potential, optimum temperature, germination index, hydro-priming period, supra optimal temperature, optimum water potential

# Introduction

Maize is a major cereal crop in the world that is on number three in ranking after wheat and rice in terms of production. Maize (*Zea mays* L.) is an important multipurpose cereal crop used as food, feed, fodder, fuel and in the manufacture of industrial products. Maize is widely adapted to variable agro-ecological conditions all over the world (Dlamini 2015). (Hu, Fan, et al. 2015) indicate that the sequencing of germination is clearly linked to temperature and water potential thresholds for radicle emergence, which vary among individual seeds in a population. For Hydrothermal time (HTT) models can estimate how any specific fraction of a seed population will respond to changes in environmental conditions (Bradford 2002, Horn, Nettles, et al. 2015), these models can also be applied to analyse a reduction in germination of the rare prairie annual *Cryptantha minima* (Yang, Li, et al. 2014). Knowledge of what causes PANNAR maize cultivars to germinate differently in the same environmental conditions is not readily available. Little information is available as to why one cultivar performed better compared to the other two cultivars in terms of absolute total germination percentage and seedling growth under the same abiotic factors e.g. temperature and water potential.

(Dumont, Andueza, et al. 2015) the response to temperature depends on a number of factors, including the species, variety, growing region, quality of the seed, and duration from harvest. As a general rule, temperate-region seeds require lower temperatures than do tropical region seeds, and wild species have lower temperature requirements than domesticated plants. High-quality seeds are able to germinate under wider temperature ranges than low-quality seeds.

The main objective of the study was to determine performance of maize cultivars by applying hydrothermal time (HTT) model to establish the germination characteristics and their response to changes in water availability and temperature conditions. The specific objectives were to: (1) construct a hydrothermal time model for 3 PANNAR maize seeds and establish important germination thresholds and parameters; (2) examine whether fluctuating temperatures and water potentials stimulate PANNAR maize seeds germination and (3) based on the parameters obtained from the HTT model, discuss the effects of climate change, specifically of increasing temperature and water potential on the PANNAR maize seed germination.

## 1.1.1. Effect of temperature on maize seed germination and seedling growth

Temperature is known to be one of the major environmental factors that control maize seed germination directly. This is because of the role it plays in breaking seed dormancy and climatic conditions alterations. Temperature regulates both germination percentage and germination rate (Li, Khan, et al. 2015). Most importantly, there are three ways temperature controls germination. These are: regulating the capacity and the rate of germination, removal of primary and secondary dormancy and lastly it induces secondary dormancy (Hawkins 2014). Above the ideal level, where germination rate is more rapid, a decline occurs as the temperature gets to lethal limit as seed is injured. Germination percentage, unlike germination rate, may remain relatively constant, at least over the middle part of temperature range if enough time is allowed for germination to take place (Nuugulu 2013). Maize seed germination which is a combination of several individual reactions and phases is affected by temperature. Effect of temperature on germination can be put as cardinal temperature: that is minimum, optimum and maximum temperatures at which germination takes place. Minimum temperature is problematic to state as germination may actually be proceeding at such a low rate that determination of germination is often made before actual germination is completed. Optimum temperature is the temperature giving the greatest percentage of germination in the shortest time frame. Maximum temperature is one at which no germination is recorded and is characterized by denaturing of proteins essential for germination. Most researchers concur that optimum temperature for germination of most seeds is between 15 and 30°C (Shaban 2013). Each germination and seedling growth stage has got its own cardinal temperature and this lead to temperature changes throughout the germination period because of the complexity of the germination process. The rate of plant development for any maize hybrid is directly related to temperature, so the length of time between the different stages will vary as the temperature varies, both between and within growing seasons.

Temperature is key factor determining germination in the arid and semiarid areas (Florentine, Weller, et al. 2016). (Bewley, Bradford, et al. 2013) pointed that maximal germination can occur over a range of temperatures and germination decreases sharply on either side of the range. Germination rate usually increases linearly with increasing temperature from minimal to an optimum and decreases linearly to a ceiling temperature (Andrade and Cardoso 2016). Germination rate rises linearly with temperature at the sub-optimal temperature range (temperature between  $T_b$  and  $T_o$ ), which is a result of

thermal dynamics. Suggested causes of fall in GR at supra-optimal temperature range ( $T_o$  and  $T_c$ ) are thermal denaturation of proteins, membrane dysfunction and interactions with water (Lukeš, Procházková, et al. 2014).

### 1.1.2. Effect of water potential on maize seed germination and seedling growth

Many factors determine flow of water from the soil into the seed and of these, water relationship between seed and soil is the major one. Water potential ( $\psi$ ) is an expression of the water energy status where the net diffusion of water occurs down an energy gradient from high to low water potential. In the seed, three factors determine the water potential and these are: osmotic potential ( $\psi_\pi$ ), pressure potential ( $\psi_p$ ) and matrix potential ( $\psi_c$ ) (Nuugulu 2013). The total ( $\psi_\pi + \psi_p + \psi_c$ ) of the three terms determines water potential. Basically  $\psi_\pi$  is determined by the concentration of dissolved solutes in water or the cell,  $\psi_c$  particles or cell wall, starch and bodies and their ability to absorb water and  $\psi_p$ , by internal pressure built up in a cell which exerts a force on the cell wall. The three terms (osmotic potential, pressure potential and matrix potential) normally result in negative form except in a situation where it approaches zero –(Leitner, Meunier, et al. 2014).

Difference in water potential between seed and soil is one of the major factors determining availability and the rate of water flow into the seed (Lobet, Couvreur, et al. 2014). At the beginning the difference in water potential between the dry seed and moist germination medium is rather large because of higher matrix. (Zaritzky 2015) stated that as seed moisture content increases during imbibitions and the tissue becomes hydrated, the water potential of the seed increases (becoming less negative). It is crucial to note that the movement of water into the seed is largely influenced by the properties of the seed and by the environment in which the seed is situated. Water potential gradient between the seeds and its surroundings is a driving force for water uptake, but the permeability of the seed to water is more important in determining its rate of uptake (Martínez-Ballesta, Zapata, et al. 2016). Seed permeability is influenced by morphology, structure, composition, initial moisture content and temperature at imbibition. The rate of water uptake is not necessarily influenced by one of the above mentioned events, but their complex interaction (Obidiegwu, Bryan, et al. 2015). Water is critical for seed germination and seedling growth. Mostly, GR increases linearly with water availability and germination percentage is reduced at reduced water potential ( $\psi$ ). Water has more complicated effects on

germination than temperature, especially at low  $\psi$ . When  $\psi$  is lower than  $-0.5$  MPa, physiological adjustment occurs. Final germination percentage remains constant after approaching a lower level at reduced  $\psi$ , rather than gradually increasing over time as it does at low temperature (Edwards, Burghardt, et al. 2016). The minimal water potential for seed germination, base water potential moves with seed physiological status, dormancy and imbibition environmental conditions (Moral, Lozano-Baena, et al. 2015). Sensitivity of seeds to water availability can change during germination. Seeds are capable of germinating at high levels of water stress and at optimum temperatures. Water requirements for seed germination i.e.  $\psi_b$ , increase with increasing temperature at supra-optimal temperature range in potato seed, carrot seed and onion seed (Soltani, Soltani, et al. 2013). Relationship between GR and temperature can be modified by water availability.

### 1.1.3. Physiology of seedling development

Abiotic stress such as drought and extreme temperatures are serious threats to crop production and food security in general. Temperature and water deficit stress are said to be the primary cause of crop failure throughout the globe (Shafique, Rehman, et al. 2014). Drought and temperature stress are known to be responsible for physiological, morphological, biochemical and molecular changes that affect seedling growth (Hasanuzzaman, Nahar, et al. 2013). Maize cultivars develop different mechanisms to cope with drought and extreme temperature stress. The plants close their stomata when they experience water shortage to prevent further loss of moisture through transpiration (Moshelion, Halperin, et al. 2015).

### 1.1.4. Germination and Emergence (VE)

Under desirable field conditions, the planted seed absorbs water and begins growth. The radicle is first to begin elongation from the swollen kernel, followed by the coleoptile with the enclosed plumule (embryonic plant), and then the three to four lateral seminal roots (Gianinetti 2016). VE (emergence) is finally attained by rapid mesocotyl elongation which pushes the growing coleoptile to the soil surface. Under warm, moist conditions, plant emergence will occur within 4 to 5 days after planting, but under cool or dry conditions, 2 weeks or longer may be required –(Wright, Schnitzer, et al. 2015).

### 1.1.5. Drought Stress

For plants to adapt to warm or low moisture environment they require the ability to live under moderate to extreme drought and heat stress (Horton, Mankin, et al. 2016). The strategy to achieve this may involve either water and heat stress avoidance or tolerance. The gene expression in tolerant plants may cause alteration of nucleic acid structures (point mutation) and/or proline metabolism or both (Filippou, Antoniou, et al. 2016, Hirayama and Shinozaki 2010). Changes in proline (free amino acid) content in plants under stress have often been used as a marker for one or other activated tolerance mechanism (Minocha, Majumdar, et al. 2014). The clear grasping of changes in protein profiles during abiotic stress may improve our understanding of drought tolerance and maize crop responsiveness to stress (Ng, De Clercq, et al. 2014). The effect of drought stress on protein content or profile is reported in soybean (Ng, De Clercq, et al. 2014). It is further stated a strong correlation exists between the synthesis of so called heat shock proteins (HSPs) and thermo tolerance (Lin, Chai, et al. 2014). It has been widely assumed that the purpose of the heat shocks response (HSR) is to protect organisms from the detrimental effects of heat and/or drought stress (Carnemolla, Labbadia, et al. 2014). A common exhibition of heat shock proteins (HSRs) was reported in amaranth, maize and potato plants under stress (Huerta-Ocampo, Barrera-Pacheco, et al. 2014).

### 1.1.6 Hydrothermal Model

The effects of variations in moisture levels (water potential) and temperatures can be described by use of hydrothermal time models (Mesgaran, Mashhadi, et al. 2013). These models characterize seed germination rates and/or seedling growth by linking them to the amounts by which temperature (T) and water potential ( $\psi$ ) are above base or threshold values for the particular plant species/cultivar (Han 2015). The timing of germination is critical, as the likelihood of seedling is dependent upon the subsequent availability of adequate water, temperature, light and nutrients to support plant growth. From the hydrothermal model, an individual plant begin to germinate or flower when the total of differences between mean daily temperature and a minimum temperature  $T_{min}$ , accumulated over many days, passes through  $\psi_T$  (measured in degree-days) i.e.  $\sum(T-T_{min}) > \psi_T$  or  $(T-T_{min})t > \psi_T$  for constant T. The HTT model increases the temperature sum with the accumulation of soil moisture potential per degree day (Werle, Sandell, et al.

2014). Seeds are believed to germinate when they have been put to efficient moisture and temperature i.e. when hydrothermal conditions have stayed for the required hydrothermal time  $\psi_{HT}$ . Hydrothermal time is found by adding the product of differences of daily moisture ( $\Psi_{soil}$ , measured as soil water potential), and temperature ( $T_{soil}$ ) to their respective base or minimum values ( $\Psi_{min}, T_{min}$ ):

$$\Psi_{HT} = \sum[(\Psi_{soil} - \Psi_{min}) \cdot (T_{soil} - T_{min})] \dots \dots \dots \text{Eq. 1}$$

With  $\Psi_{soil} > \Psi_{min}$  and  $T_{soil} > T_{min}$ .

Hydrothermal time and minimum temperature are assumed constant for a species, whereas minimum moisture is assumed normally distributed within a population of seeds (Atashi, Bakhshandeh, et al. 2015) with a mean  $\Psi_{min50}$  and its standard deviation  $\sigma(\Psi_{min})$ .

Germination rate, the time until a certain fraction of seeds has germinated, can be calculated by specifying g and the HTT parameters. The mean minimum moisture  $\Psi_{min50}$  seem to vary more or less strongly with temperature in many species (Ooi, Denham, et al. 2014) and has also been shown to vary among seed lots (Ooi, Denham, et al. 2014). The four parameters  $\Psi_{HT}, \Psi_{min50}, T_{min}$  and  $\sigma(\Psi_{min})$  are mostly determined by repeated probit regression or by non-linear regressions of data from germination experiments at factorial combinations of several levels of constant soil moisture and temperature (Abedi 2013). The hydrothermal time model proposes that seed germination rates are proportional to the amount by which temperature (T) and water potential exceed base or threshold values for those environmental factors (Barth, Meyer, et al. 2015). Role of temperature is to determine the rate of progress toward completion of germination once a non-dormant seed is stimulated to germinate. The germination rate for a given seed fraction (GRg) is often a linear function of temperature between base temperature ( $T_b$ ) i.e. lowest temperature below which no germination is recorded and optimum temperature ( $T_o$ ) temperature at which rapid and majority of seeds in the seed population germinate (Hay, Mead, et al. 2014). Hydrothermal time model is a result of the combination of thermal time and hydro time that describe seed germination patterns.

This study aimed at identifying the best cultivar to be grown by farmers under varying hydrothermal conditions reducing risks of climate change in South Africa. In determining the effects on germination and seedling growth, the results of the study hope to assist smallholder farmers choose the most suitable cultivar for their area. The specific objective of this chapter was to establish the differences in maize cultivar in terms of response to drought and heat stress at germination

## 1.2. Materials and Methods

Parameters of the hydrothermal time model were established from the relationships between germination rate (GR) and temperature at various water potentials ( $\psi$ ) and between GR and  $\psi$  at various temperatures. Model assumptions were tested using these parameters. This investigation was performed as a factorial experiment under randomized complete block design (RCBD) with three replicates. Water gradients were established using polyethylene glycol (PEG-6000, EMC, Germany) solutions (Zhang, Wang, et al. 2015). Germination rates of subpopulations were estimated from germination time courses over five water potentials of:

-0.05MPa  
-0.1MPa  
-0.2MPa  
-0.4MPa  
-0.6MPa

And 5 temperatures of:

15°C  
20°C  
25°C  
30°C  
35°C

The water potentials of PEG solutions were measured using a vapor pressure osmometer (Model 5100, Wescor Inc. Logan, UT). The measurements were collected 30 min after PEG solutions penetrated into two layers of filter papers (Whitman No. 1) in Petri dish as suggested by (Zhang, Xie, et al. 2016) to take effect of filter paper on water potential into consideration. Value of water potential was adjusted to suit each germination temperature for modeling. Germination tests were conducted in darkness using five incubators. Designated temperatures 15, 20, 25, 30 and 35°C, were randomly allocated to each incubator.

A randomized complete block design (RCBD) with two runs was used and the second run was started 21 days after the first run. Seeds, 40/unit, were placed in 9 cm Petri dishes with distilled water or PEG solutions on top of two layers of filter papers (Whitman No. 10) and Petri dishes were randomized within incubator shelves. Five mL of PEG solution were added initially to each Petri dish and an extra 2 mL were added after 24 h and periodically as required. Clear plastic bags were used to seal Petri dishes to reduce water evaporation. The maize seeds were considered germinated when either the emerging radicle or cotyledon was =2 mm. Germination was recorded daily for up to 10 days

in 2 consecutive days in all Petri dishes. Germinated seeds and rotten seeds were removed after each counting. Germination percentages were averaged over the three replicates before continuing with analysis. The percentage of viable seeds was estimated at the end of the germination test and adjusted to a scale of 0-100% by dividing final germination percentage with a scaling factor (Burghardt 2015).

### 1.2.1. Parameter estimation for the hydrothermal time model

The experiment included a full factorial design of treatments derived from three factors: 3 cultivars, 5 water potentials and 5 temperatures. Water potential levels were:

-0.05Mpa  
-0.1Mpa  
-0.2Mpa  
-0.4Mpa  
-0.6Mpa

These represented decreasing levels of water availability for seed germination and constant temperatures used were:

15°C  
20°C  
25°C  
30°C  
35°C

The range of temperatures included the temperatures the maize seeds experience in the field during germination period. Each treatment had three replicates. To determine the optimum germination temperature (i.e. the temperature where germination rate is at its maximum) a plot of germination rate versus temperature was drawn. Least squares regression lines were fitted to the 10<sup>th</sup> and 20<sup>th</sup> percentiles above and below the visually estimated optimum temperature ( $T_o$ ), which was between 20 and 30 °C. Optimum temperature per percentile was estimated as the point where regression lines above and below the visually estimated  $T_o$  crossed each other. The germination modeling was based on the HTT models (Barth, Meyer, et al. 2015) which assume that the base temperature ( $T_b$ ) and the hydrothermal time required for germination ( $\Theta_{HT}$ ) are constants, while the base water potential ( $\psi_b$ ) varies according to a normal distribution and is characterized by its mean ( $\psi_{b(50)}$ ) and standard deviation ( $\psi_b$ ) (Rong, Li, et al. 2015). The models also assume that below the optimum temperature ( $T_o$ ),  $\psi_b$  is independent of

temperature, and that the  $T_b$  is independent of water potential (Al-Mulla, Huggins, et al. 2014). Above  $T_o$ , where it is assumed that the hydrothermal time accumulation is maximal,  $\psi_b$  increase with increasing temperature with a slope equal to  $k_T$  (Barth, Meyer, et al. 2015). The modeling was performed using repeated probit analyses (Hay, Mead, et al. 2014) where the response variable, percentage of germination, was transformed to the probit scale using the PROBIT function in SAS (Rong, Li, et al. 2015). Values below 10% and above 90% of the final germination, considered data points that did not add any germination, as well as any observation where no increase in germination percentage occurred, were therefore excluded. Goodness of fit of models was checked by obstructing plots of germination percentage versus the normalized thermal time (Khorsand Rosa, Oberbauer, et al. 2015).

### 1.2.2. Germination modeling

To estimate the GR (g), germination time courses of each temperature, water potential, and replicate were fitted separately using probit analysis procedure. The tg for subpopulation was estimated using linear function of  $GR_{(50)}$  on temperature for each water potential. The linear function of  $GR_{(50)}$  on water potential was used to estimate  $t_g$  at each germination temperature. Similarly, the linear functions of specific g fraction on  $\psi$  for subpopulation:

- 10%
- 20%
- 30%
- 40%
- 50%
- 60%
- 70%
- 80%

were used for  $\Theta_H$  estimation. Data from all replicates and the regressions were disregarded when they were not significant ( $P > 0.05$ ).

### 1.2.3. Comparison of hydrothermal time models based on different assumptions

The basic hydrothermal time model was based on (Atashi, Bakhshandeh, et al. 2015) hydrotime constant,  $H$ , is calculated as:

$$H = (\Psi - \Psi_b(g))tg \dots \dots \dots 1.2$$

where  $\Psi$  is the actual water potential, and  $\Psi_b(g)$  is the minimal water potential or base water potential

that prevents germination of percentage g.

Hydrothermal time,  $HT$ , is:

$$\Psi HT = (T - T_b) (\Psi - \Psi_b(g))tg \dots \dots \dots 1.3$$

According to the assumptions of the hydrothermal time model, germination progress can be described by the normal distribution of  $\psi_{b(g)}$  within a seed population in Eq 1.3. (Wang, Bai, et al. 2005). The 50% subpopulation ( $\Theta_{HT(50)}$ ) was used in the hydrothermal time model for simplification even though variation existed among subpopulations. The predictability of the hydrothermal time model based on different assumptions was tested 1):  $T_b$  and  $\Theta_{HT}$  were assumed to be constant within a seed population (Atashi, Bakhshandeh, et al. 2015). Base temperature of the 50% subpopulation ( $T_{b(50)}$  at MPa) was used as the common  $T_b$  for the seed population. Daily germination rate was measured and filter papers were replaced when needed. Seeds were considered germinated when the emergent radical reached 2mm length. After 10 days germination index and seedling vigour index was measured by International Seed Testing Association (Vashisth and Joshi 2016).

## 1.3 Results

### 1.3.1 Final germination percentage

Cumulative maize seed germination was significantly affected by temperature. Cultivar, temperature and water potential had significant impact on cumulative maize seed germination (fig. 1.1a & 1b). PAN413 had the highest cumulative germination percentage at all temperatures. The final germination percentage of maize seed was significantly influenced by cultivar, temperature, water potential and cultivar x temperature (Table 1.1), cultivar x water potential (fig 1.2.) temperature x water potential (fig 1.3) and cultivar x temperature x water potential (Table 1.3) interactions. The significant effect of temperature and water potential interaction shows that there is interdependence between these two factors for the germination of maize seed (fig 1.4). Observations from fig 1.4, show that a rise in temperature and a fall in water potential significantly lowered final germination percentage of maize seed. The negative impact of water potential is particularly witnessed at 30°C and 35°C where significant reduction in final germination percentage occurred with each incremental fall in water potential from -0.4MPa to -0.6MPa. At 25°C there is little significant reduction in the final germination percentage as water potential decreases than at 30 and 35°C. It can also be observed

that the final germination percentage at each water potential falls as the temperature rises, with the lowest final germination percentage recorded at the highest temperature. These results show that maize seed usually germinates best at a temperature between 25 and 30°C and a water potential of 0 to -0.2MPa, although the neutral (0) water potential is affected by temperature.

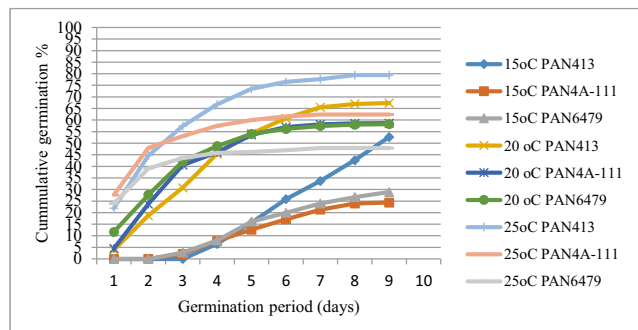


Fig.1.1a. Cumulative germination for the three cultivars at 15-25°C over 10 day period

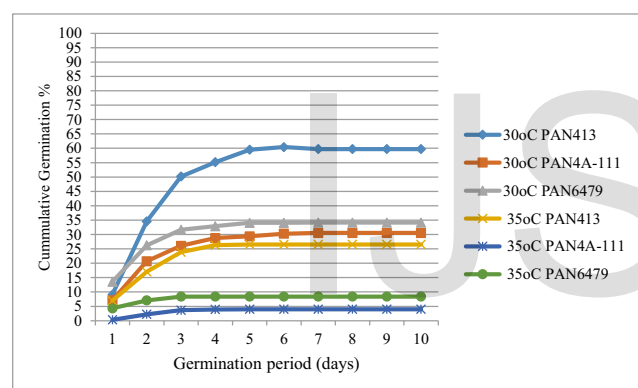


Fig. 1.1b. Cumulative germination for the three cultivars at 30-35°C over 10 day period

Table 1.1. Effect of water potential on final germination percentage

| Water potential (MPa) | N  | Mean FGP |
|-----------------------|----|----------|
| -0.05                 | 90 | 52.995a  |
| -0.1                  | 90 | 50.550b  |
| -0.2                  | 90 | 42.922c  |
| -0.4                  | 90 | 37.411d  |
| -0.6                  | 90 | 25.612e  |

\*Number with the same letters are not significantly different.

Table 1.2. Interactional effect of cultivar and temperature on final germination percentage

| Cultivar  | Temperature | N  | Mean FGP | StdDev |
|-----------|-------------|----|----------|--------|
| PAN413    | 15          | 30 | 43.17    | 18.29  |
|           | 20          | 30 | 67.67    | 19.07  |
|           | 25          | 30 | 79.58    | 14.43  |
|           | 30          | 30 | 61.00    | 10.84  |
|           | 35          | 30 | 26.58    | 14.47  |
| PAN4A-111 | 15          | 30 | 24.33    | 13.28  |
|           | 20          | 30 | 56.67    | 14.86  |
|           | 25          | 30 | 62.24    | 13.80  |
|           | 30          | 30 | 29.31    | 15.50  |
|           | 35          | 30 | 4.00     | 3.19   |
| PAN6479   | 15          | 30 | 27.32    | 16.45  |
|           | 20          | 30 | 58.15    | 11.91  |
|           | 25          | 30 | 47.90    | 13.78  |
|           | 30          | 30 | 34.15    | 11.12  |
|           | 35          | 30 | 8.42     | 7.33   |

From fig.1.2 PAN413 had the highest mean germination percentage at all the water potentials. At -0.05MPa PAN413 had MFGP of 67.83% and at -0.6MPa it had MFGP of 37.75%. PAN4A-111 was second in terms of response to interaction of cultivar and water potential. PAN 6479 had the least MFGP of 20.83% at -0.05MPa and 15.16% at -0.6MPa.

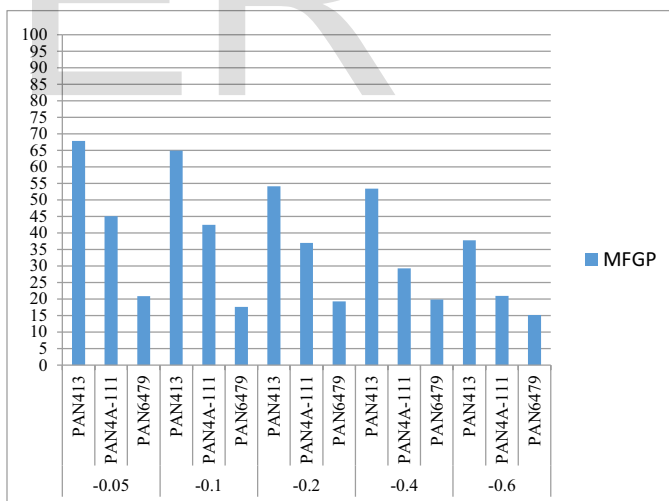


Fig 1.2 Interactional effect of cultivar and water potential on final germination percentage

Interaction effect of temperature and water potential had significant effect on final germination percentage of the three maize cultivars. Temperature of 25°C and -0.05MPa had the highest final germination percentage

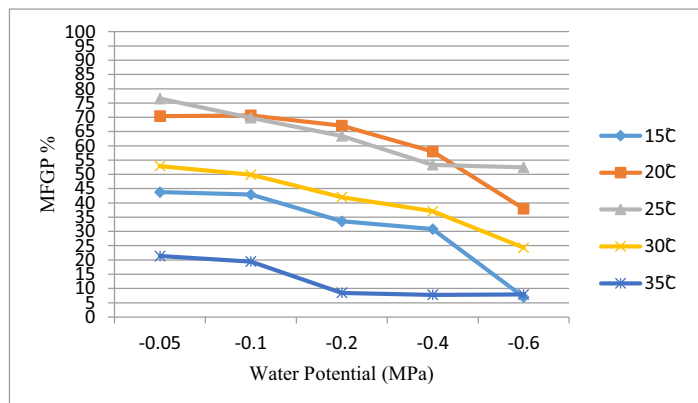


Fig.1.3 Interactional effect of temperature and water potential on final germination percentage

The optimum temperature ( $T_o$ ) for PAN 413 and PAN 4A-111 was 25°C, but for PAN 6479 it was 20°C. The models at higher water potentials performed much better as they explained more than 80% of the variation. The germination curves under normalized thermal time (i.e. a time scale with the number of °C above the base temperature for germination multiplied by the time to reach a given germination percentage) for the below and above optimum temperature showed that the models accounted for the observed germination patterns, as indicated by the overlap of the data points. The thermal time to reach 50% of the germination increased exponentially as water potential decreased with the shortest equal to 2.48days at 25 °C and -0.05MPa for PAN 413, 1.73 days at 25°C and -0.05Mpa and 2.53days at 20°C and -0.05Mpa. Above  $T_o$ , the observed ceiling temperature for the three PANNAR cultivars was 35°C with water availability equal to  $\psi=-0.6$ Mpa.

### 1.3.3. Germination modelling

The germination model shows the linear relationship of final seed germination to temperature and water potential. At low temperature of 15°C, there was low final germination percentage by all the three maize cultivars supporting the fact that each crop has its optimum germination temperature and base temperature. For maize the base temperature ( $T_b$ ) is 10°C and in this study 15°C was the lowest temperature nearer to base temperature. Base water potential for maize is -0.5MPa and in the study the lowest water potential was -0.6MPa. Looking at

fig. 3.2. followed by 20°C and -0.05MPa. As both temperature and water potential increased, final germination percentage significantly went down.

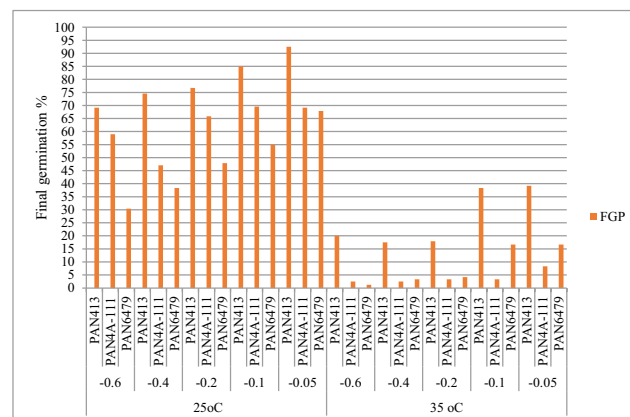


Fig.1.4 Interactional effect of cultivar “temperature” water potential on FGP of PAN4A-111

water potential alone optimum final germination percentage was observed around 0MPa and -0.2MPa for all the three cultivars. The germination model indicated that as temperature increased from 15 -25°C and water potential was -0.2 to -0.05MPa, final germination percentage increased significantly. From 30-35°C and water potentials of -0.4 to -0.6MPa final germination percentages went down

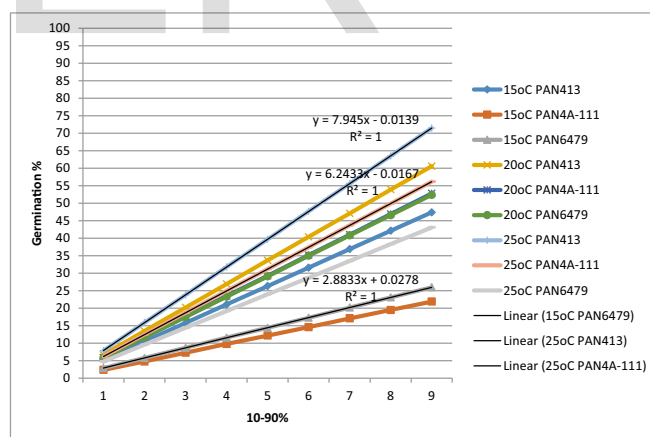


Fig.1.5a. Germination % of 10-90% of final germination percentage for the three cultivars at 15-25°C

From fig.1.5a above, the 10-90% of final germination percentage that of PAN413 at all temperatures was above the other two cultivars i.e. PAN4A-111 and PAN6479. These percentages are critical as they are used to compare how best a variety performs in relation to the other cultivars. The equations or models show the linear relationship of temperature, time and germination percentage. For example at 25°C the model for PAN413 and PAN4A-111 were as follows:



- PAN413:  $Y=7.945x-0.0139$ .....3.1
- $R^2=1$ .....3.2
- PAN4A-111:  $Y=6.2433x-0.0167$ .....3.4
- $R^2=1$ .....3.5

As temperature rose 30-35°C, the 10-90% of final germination percentage dropped. The following models indicate the fall in germination percentages.

- PAN413:  $Y=5.9683x+0.0472$ .....3.6
- $R^2=1$ .....3.7
- PAN4A-111:  $Y=3.0633x-0.0167$ .....3.8
- $R^2=0.999$ .....3.9

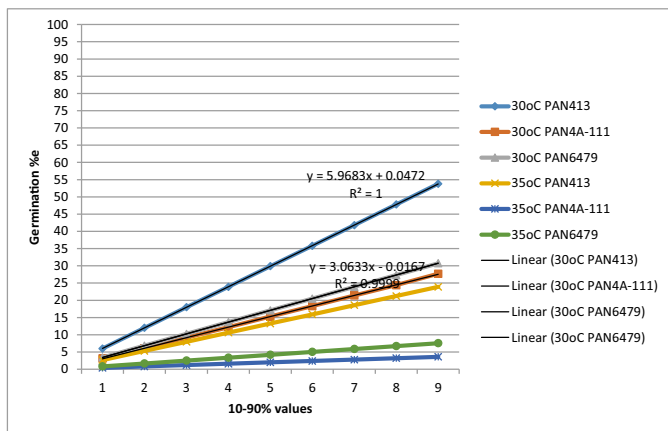


Fig.1.5b. Germination % of 10-90% of final germination percentage for the three cultivars at 30-35%

Table 1.3. 10-90% of final germination and time taken by the three cultivars over the five different temperature

| Temp | Cultivar  |             | 10% | 20%  | 30%  | 40%   | 50%   | 60%  | 70%  | 80%   | 90%  |
|------|-----------|-------------|-----|------|------|-------|-------|------|------|-------|------|
| 15°C | PAN413    | Time(days)  | 4.4 | 5.25 | 5.5  | 6.1   | 6.5   | 7.15 | 8    | 8.4   | 9.25 |
|      |           | GN%         | 5.3 | 10.5 | 15.8 | 21.07 | 26.34 | 31.6 | 36.9 | 42.14 | 47.4 |
|      | PAN4A-111 | Time(days)  | 3   | 3.5  | 3.8  | 4.3   | 5     | 5.5  | 6    | 6.5   | 7.48 |
|      |           | GN%         | 2.4 | 4.8  | 7.3  | 9.8   | 12.2  | 14.6 | 17.1 | 19.5  | 21.9 |
|      | PAN6479   | Time (days) | 3   | 3.52 | 4.2  | 4.4   | 4.8   | 5.4  | 6.3  | 7     | 7.6  |
|      |           | GN%         | 2.9 | 5.8  | 8.7  | 11.6  | 14.4  | 17.3 | 20.2 | 23.1  | 26   |
| 20°C | PAN413    | Time(days)  | 1.2 | 1.6  | 2.2  | 2.7   | 3.3   | 3.6  | 4.1  | 4.7   | 6.3  |
|      |           | GN%         | 6.7 | 13.5 | 20.2 | 26.9  | 33.7  | 40.4 | 47.1 | 53.9  | 60.6 |
|      | PAN4A-111 | Time(days)  | 1.1 | 1.4  | 1.6  | 2     | 2.4   | 2.6  | 3.1  | 4.1   | 4.9  |
|      |           | GN%         | 5.9 | 11.7 | 17.6 | 23.5  | 29.3  | 35.2 | 41   | 46.9  | 52.8 |
|      | PAN6479   | Time(days)  | 0.8 | 1.1  | 1.4  | 1.7   | 2.1   | 2.48 | 2.8  | 3.6   | 4.7  |
|      |           | GN%         | 5.8 | 11.7 | 17.5 | 23.3  | 29.1  | 35   | 40.8 | 46.6  | 52.4 |
| 25°C | PAN413    | Time(days)  | 0.7 | 0.9  | 1.1  | 1.4   | 1.8   | 2.3  | 2.9  | 3.6   | 4.7  |
|      |           | GN%         | 7.9 | 15.9 | 23.8 | 31.8  | 39.7  | 47.7 | 55.6 | 63.5  | 71.5 |
|      | PAN4A-111 | Time(days)  | 0.7 | 0.7  | 0.8  | 0.9   | 1.2   | 1.5  | 1.7  | 2.4   | 3.7  |
|      |           | GN%         | 6.2 | 12.5 | 18.7 | 25    | 31.2  | 37.4 | 43.7 | 49.9  | 56.2 |
|      | PAN6479   | Time(days)  | 0.7 | 0.8  | 0.9  | 0.9   | 0.9   | 0.9  | 1.4  | 1.8   | 3    |
|      |           | GN%         | 4.8 | 9.6  | 14.4 | 19.2  | 24    | 28.7 | 33.5 | 38.3  | 43.1 |
| 30°C | PAN413    | Time(days)  | 0.8 | 1.1  | 1.4  | 1.6   | 1.8   | 2.1  | 2.5  | 2.8   | 3.7  |
|      |           | GN%         | 6   | 12   | 18   | 23.9  | 29.9  | 35.8 | 41.8 | 47.8  | 53.8 |

|      |           |            |     |     |      |      |      |      |      |      |      |
|------|-----------|------------|-----|-----|------|------|------|------|------|------|------|
|      | PAN4A-111 | Time(days) | 0.7 | 0.9 | 1.2  | 1.4  | 1.6  | 1.8  | 2.1  | 2.7  | 3.2  |
|      |           | GN%        | 3.1 | 6.1 | 9.2  | 12.2 | 15.3 | 18.3 | 21.4 | 24.4 | 27.7 |
|      | PAN6479   | Time(days) | 0.7 | 0.8 | 0.9  | 1    | 1.4  | 1.6  | 1.7  | 2.3  | 2.7  |
|      |           | GN%        | 3.4 | 6.8 | 10.2 | 13.7 | 17.1 | 20.5 | 23.9 | 27.3 | 30.7 |
| 35°C | PAN413    | Time(days) | 0.7 | 0.8 | 1.1  | 1.4  | 1.6  | 1.8  | 2.2  | 2.6  | 3    |
|      |           | GN%        | 2.7 | 5.3 | 8    | 10.6 | 13.3 | 16   | 18.6 | 21.2 | 23.9 |
|      | PAN4A-111 | Time(days) | 0.7 | 0.7 | 0.8  | 0.9  | 1.7  | 2    | 2.7  | 3    | 3.3  |
|      |           | GN%        | 0.4 | 0.8 | 1.2  | 1.2  | 1.6  | 2.4  | 2.8  | 3.2  | 3.6  |
|      | PAN6479   | Time(days) | 0   | 0   | 0    | 1    | 1.1  | 1.2  | 1.7  | 1.8  | 2.3  |
|      |           | GN%        | 0.8 | 1.7 | 2.5  | 3.4  | 4.2  | 5.0  | 5.9  | 6.7  | 7.6  |

Time taken to reach certain percentages of the final germination percentages decreased as temperature increased e.g. at 15°C PAN413 4.4days to have 10% of final germination percentage but at 35°C it only needed 0.7days to get to the same 10% of final germination percentage. To reach 50% of final germination percentage, PAN413 required 1.8days, PAN4A-111 required 1.2days and PAN6479 needed 0.9days at 25°C.

### 1.3.4. Time to 50% of final germination percentage

$T_{50}$  is time taken by a cultivar of maize to reach 50% of the final germination percentage under the different hydrothermal conditions in this case under set temperature and water potentials. From table 3.2 PAN413 required more time to reach 50% of the final germination at all different five temperatures and five water potentials. PAN6479 had the lowest time to reach 50% of final germination percentage. There was no linear relationship between  $T_{50}$  and final germination percentage. The cultivar with the longest period to reach  $T_{50}$  finally had the highest germination percentage but the one with lowest  $T_{50}$ , ended with the lowest final germination percentage. Water potential had significant effect on mean  $T_{50}$  at -0.05MPa and -0.6MPa. The observed trend was that  $T_{50}$  decreased with a decrease in water potential. Under low water potential, maize seed took less time to get to 50% of the final germination percentage.

Table 1.4 Effect of water potential on  $T_{50}$

| Water potential (MPa) | N  | Mean $T_{50}$ |
|-----------------------|----|---------------|
| -0.05                 | 90 | 2.8444a       |
| -0.1                  | 90 | 2.7629ab      |
| -0.2                  | 90 | 2.6406ab      |
| -0.4                  | 90 | 2.5945ab      |
| -0.6                  | 90 | 2.5156b       |

\*Numbers with the same letters are not significantly different

### 1.3.5 Germination rate index

Germination rate index was significantly influenced by cultivar and temperature interaction. At 15°C all the three cultivars had very low germination rate index (Fig. 3.6b). As temperature increased from 15°C to 25°C, the GRI also increased at an increasing rate (Fig. 3.6b). For PAN413 it continued increasing at a decreasing rate until it got to 30°C where the highest GRI was recorded. Highest value of GRI was observed under 25°C for all the three cultivars. The cultivar with the highest GRI indicated that it had the highest GRI energy and is therefore a better cultivar under varying temperature and water potential. The result showed that highest germination index was observed in PAN413 and germination index was considerably reduced under high temperature and low water potential condition (Fig. 1.6a and Fig. 1.6b). Under high temperature and low water potential maize plants showed highest percentage 43.34% and 30.68 germination index. Highest germination index was observed at four days as compared to seven and ten days after sowing.

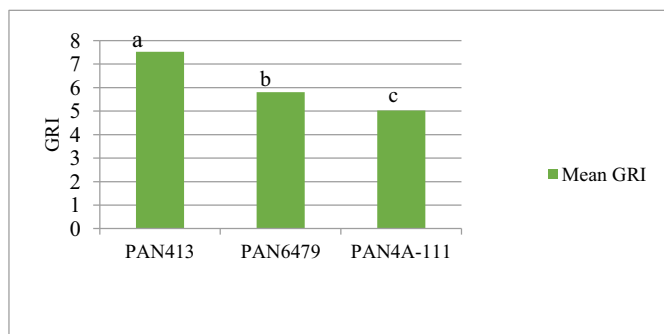


Fig. 1.6a Effect of cultivar on GRI

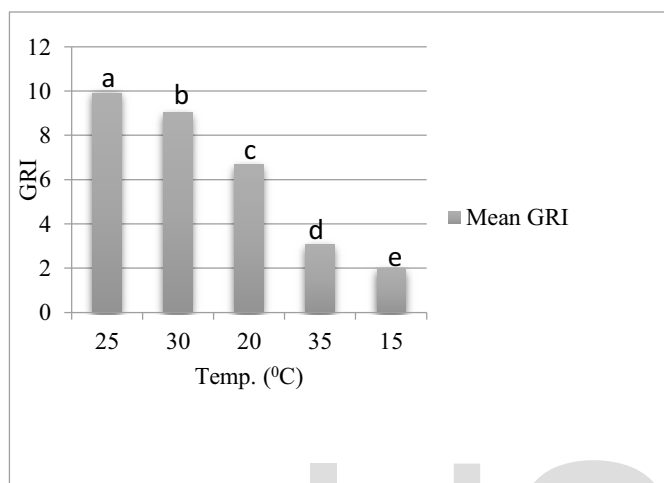


Fig. 1.6b Effect of temperature on GRI

### 1.4 Discussion

The study was able to establish that temperature and water potential played an important role in maize seed germination as seen in the decrease in the final percentage germination rate with increases in the temperature regimes and decrease in water potential. (Essemine, Ammar, et al. 2010) reported that inhibition of seed germination with increase in temperature often occurs through induction of ascorbic acid (ABA). Seeds exposed to very high temperatures and low water potential in this study must have produced more ABA thus inhibiting germination (Piskurewicz and Lopez-Molina 2016). At very high heat stress (35°C) and low water potential of -0.6MPa, the rate of maize seed germination was strictly prohibited and caused cell death and embryos for which seedling establishment rate was also reduced (Kumar 2014). In this study, it can be concluded that low water potential induced water stress adversely affected the germination of maize varieties. Distinct genetic differences were found among the varieties with respect to final germination percentage, time to reach 50% of final germination percentage and germination rate index when exposed to high temperatures and low water potential. Low water potential is physiologically related. PAN413 in high temperatures and low water potential condition was more tolerant compared to PAN4A-111 and PAN6479. Low water potential

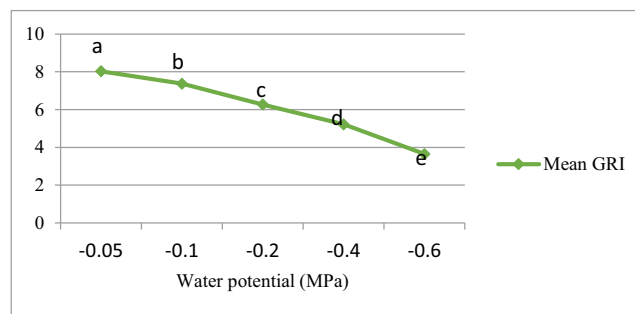


Fig.4.7 Effect of water potential on GRI

significantly reduced final germination percentage,  $T_{50}$  and germination index (Lewandrowski, Erickson, et al. 2016). Under high temperature and low water potential, germination was reduced as a result of shortage of water required for early processes of germination. Low water potential had a lethal effect on germinating seeds. High range of temperature resulted in reduction in seed germination rate because high temperature affects enzyme functions and initiates moisture stress. In the study, negative effects of low water potential were found on final germination percentage,  $T_{50}$  and germination rate index. Reason behind this effect may be the disturbance in the physiology due to increase in osmotic stress (Dubey, Srivastava, et al. 2014). Previous studies suggested that low water potential can contribute to improved germination rate and seedling emergence in different plant species by strengthening presence of aquaporins (Galhaut, de Lespinay, et al. 2014), by increase of amylases and proteases or lipases activity (Galhaut, de Lespinay, et al. 2014). Soil moisture deficiency due to drought is most likely the significant abiotic factor limiting seed germination and seedling

Growth (Avramova, Nagel, et al. 2016). Results of the study reveal that PAN413 had tolerance to water stress conditions as compared to PAN4A-111 and PAN6479. Maize seeds required 7-10 days to germinate. The long range was as a result of varying temperatures and water potential levels used in the study. Duration to germination varied as much as 3-4 folds within the range of temperature conditions used. In general, the duration of germination was the shortest at temperatures between 25 and 30°C and was higher at temperatures outside the range. Duration of germination increased with the decreasing temperature and water potential. Seeds did not germinate under treatment conditions of 35°C and -0.6MPa water potential treatment. Predicted cumulative germination percentages over time at various water potential levels ( $\psi$ ) and temperature regimes generally matched well with observations. Estimated values of  $\psi_b$  (50), and  $\theta_H$  were specific for each temperature regime.

# References

- [1.] Abedi M. 2013. Seed ecology in dry sandy grasslands-an approach to patterns and mechanisms.
- [2.] Al-Mulla Y A, Huggins DR, Stockle CO. 2014. Modeling the emergence of winter wheat in response to soil temperature, water potential and planting depth. *Trans. ASABE*, 57:761-775.
- [3.] Andrade L, Cardoso V. 2016. Does thermal time for germination vary among populations of a tree legume (*Peltophorum dubium*)? *Brazilian Journal of Biology*, 76: 592-599.
- [4.] Atashi S, Bakhashandeh E, Mehdipour M, Jamali M, Da Silva JAT. 2015. Application of a hydrothermal time seed germination model using the Weibull distribution to describe bas water potential in zucchini (*Cucurbita pepo L.*) *Journal of Plant Growth Regulation*. 34: 150-157.
- [5.] Avramova V, Nagel KA, AbdElgawad H, Bustos D, DuPlessis M, Fiorani F, Beemster GT. 2016. Screening for drought tolerance of maize hybrids by multi-scale analysis of root and shoot traits at the seedling stage. *Journal of experimental botany*: erw055
- [6.] Barth CW, Meyer SE, Beckstead J. Allen 2015. Hydrothermal time models for conidial germination and mycelia growth of the seed pathogen *Pyrenophora semeniperda*. *Fungal biology*. 119: 720-730
- [7.] Bewley JD, Bradford KJ, Hilhorst HW, Nonogaki H. 2013. Environmental regulation of dormancy and germination. *Seeds*: Springer. P 299-399.
- [8.] Bradford KJ. 2002. Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. *Weed Science*, 50: 248-260.
- [9.] Brughardt LT. 2015. The Influence of Genetic and Environmental Factors on the Phenology and Life—Cycle, Duke University.
- [10.] Carnemolla A, Labbadia JP, Lazell H, Neuder A, MOussaoui S, Bates GP. 2014. Contesting the dogma of age-related heat shock response impairment: implications for cardiac-specific age related disorders. *Human molecular genetics*: ddu073.
- [11.] Dlamini JC. 2015 Maize growth and yield as affected by different soil fertility regimes in a long term trial, University of Pretoria.
- [12.] Dubey RS, Srivastava RK, Pessaraki m. 2014. Physiological mechanisms of nitrogen absorption and assimilation in plants under stressful conditions. *Handbook of Plant and Crop Physiology*, Third Edition: CRC Press. P. 453-486.
- [13.] Dumont B, Andueza D, Niderkorn V, Luscher A, Porqueddu C, Picon-Cochard C. 2015. A meta-analysis of climate change effects on forage quality in grasslands: specificities of mountain and Mediterranean areas. *Grass and Forage Science*, 70:239-254.
- [14.] Edwards BR, Brughardt LT. Zapata –Garcia M, Donohue K. 2016. Maternal temperature effects on dormancy influence germination responses to water availability in *Arabidopsis thaliana*. *Environmental and Experimental Botany*, 126:55-67.
- [15.] Essemime J, Ammar S, Bouzid S. 2010. Physiological, Biochemical and Molecular Repercussions and Mechanisms of Defence. *Journal of Biological Sciences*, 10:565-572.
- [16.] Filippou P, Antoniou C, Obata T, Van Der Kelen K, Harokopos V, Kanetis L, Aidinis V, Van Breusegem F, Fernie AR, Fotopoulos V. 2016. Kresoxim-methyl primes *Medicago truncatula* plants against abiotic stress factors via altered reactive oxygen and nitrogen signalling leading to downstream transcriptional and metabolic readjustment. *Journal of experimental botany*, 67:1259-1274.
- [17.] Florentine Sk. Weller S, Graz PF, Westbrooke M, Florentine A, Javaid M, Fernando N, Chauhan BS, Dowling K. 2016. Influence of selected environmental factors on seed germination and seedling survival of the arid zone invasive species tobacco bush (*Nicotiana glauca R. Graham*) *The Rangeland Journal*, 38: 417-425.
- [18.] Galhaut L, de Lespinay A, Walker DJ, Bernal MP, Correal E, Lutts S. 2014. Seed priming of *Trifolium repens L.* improved germination and early seedling growth on heavy metal-contamination soil. *Water, Air & Soil Pollution*, 225:1905.

- [19.] Gianinetti A. 2016. Anomalous germination of dormant dehulled red rice seeds provides a new perspective to study the transition from dormancy to germination and to unravel the role of the caryopsis coat in seed dormancy. *Seed Science Research*, 26:124-138
- [20.] Han S. 2015. Salmonella enteric interactions with tomato: plant genotype effects and Salmonella genetic responses.
- [21.] Hasanuzzaman M, Nahar k, Alam MM, Roychowdhury R, Fujita m. 2013. Physiology biochemical and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences*, 14:9643-9684
- [22.] Hawkins KK. 2014. Secondary dormancy and summer conditions influence outcomes in the Pyrenophora semeniperda-Bromus tectorum pathosystem.
- [23.] Hay FR, Mead A, Bloomberg M. 2014. Modeling seed germination in response to continuous variables: use and limitations of probit analysis and alternative approaches. *Seed Science Research*, 24: 165-186.
- [24.] Hirayama T, Shinozaki K 2010. Research on plant abiotic stress responses in the post-genome era: Past, Present and future. *The Plant Journal* 61:1041-1052
- [25.] Horn KJ, Nettles R, Clair SBS. 2015 Germination response to temperature and moisture to predict distributions of the invasive grass red brome and wildfire. *Biology Invasions*, 17: 1849-1857.
- [26.] Horton RM, Mankin JS, Lesk C, Coffel E, Raymond C. 2016 A review of Recent Advances in Research on Extreme Heat Events. *Current Climate Change Reports*. 2:242-259.
- [27.] Hu XW, fan Y, Baskin CC, Baskin JM, Wang YR. 2015. Comparison of the effects of temperature and water potential on seed germination of Fabaceae species from desert and subalpine grassland. *American journal of botany*. 102:649-660
- [28.] Huerta-Ocampo JA, Barrera-Pacheco A, Medmnoza-Hernandez CS, Espitia-Rangel E, MockH-P, Barba de la AP. 2014 Salt stress-induced alterations in the root proteome of *Amaranthus cruentus* L. *Journal of proteome research*, 13: 3607-3627.
- [29.] Khorsand Rosa R, Oberbauer SF, Starr G, Parker La Puma I, Pop E, Alhquist L, Baldwin T. 2015 . Plant phenological responses to a long-term experimental extension of growing season and soil warming in the tussock tundra of Alaska. *Global change biology*. 21:4520-4532
- [30.] Kumar A. 2014 Evaluation of seed vigour parameters for heat tolerance in bread wheat, CCSHAU. Leitner D, Meunier F, Bodner G, Javaux M, Schnepf A. 2014 Impact of contrasted maize root traits at flowering on water stress tolerance – A simulation study . *Field crops Research*, 165: 125-137.
- [31.] Lewandowski W, Erickson TE, Dixon KW, Stevens JC. 2016. Increasing the germination envelope under water stress improves seedling emergence in two dominant grass species across different pulse rainfall events. *Journal of applied Ecology*.
- [32.] Li W, Khan MA, Yamaguchi S, Liu X. 2015. Hormonal and environmental regulation of seed germination in salt cress (*Thellungiella halophila*). *Plant growth regulation*. 76: 41-49.
- [33.] Lin M-y, Chai K-h, Ko S-s, kuang L-v, Lur H-S, Charng Y-y. 2014. A positive feedback loop between HEAT SHOCK PROTEIN 101 and HEAT STRESS –ASSOCIATED 32-KD PROTEIN modulates long-term acquired thermo tolerance illustrating diverse heat stress response in rice varieties. *Plant Physiology*. 164:2045-2053.
- [34.] Lobert G, Couvreur V, Meunier F, Javaux M, Draye X. 2014. Plant water in drying soils. *Plant Physiology*, 164: 1619-1627
- [35.] Lukes m, Prochazkova L, Shmidt V, Nedbalova I, kaftan D. 2014. Temperature dependence of photosynthesis and thylakoid lipid composition in the red snow alga *Chlamydomonas cf. nivalis* (Chlorophyceae) *FEMS microbiology ecology*, 89: 303-315.
- [36.] Martinez-Ballesta MC, Zapata L, Chalbi N, Carvajal M. 2016 Multiwalled carbon nanotubes enter broccoli cells enhancing growth and water uptake of plants exposed to salinity. *Journal of nanobiotechnology*, 14:42
- [37.] Mesgaran M, mashhadi H, Alizadeh H, Hunt J, Young K, Cousens R. 2013 Importance of sistribution function selection for hydrothermal time models germination. *Weed Research*, 53: 89-101.
- [38.] Minocha R, Majumdar R, Minocha SC. 2014. Polyamines and abiotic stress in plants: a complex relationship. *Plant Polyamines in stress and development*: 6.

- [39.] Moral J, Lozano-Baena MD, Rubiales D. 2015. Temperature and water stress during conditioning and incubation phase affecting *Orobanche crenata* seed germination and radical growth. *Frontiers in plant science*, 6: 408.
- [40.] Moshelion M, Halperin O, Wallach R, Oren R, Way DA. 2015. Role of aquaporins in determining transpiration and photosynthesis in water-stressed plants: crop water use efficiency, growth and yield. *Plant, cell & environment*, 38: 1785-1793.
- [41.] Ng S, De Clercq I, Van Aken O, Law SR, Ivanova A, Willems P, Girud E, Van Breusegem F, Whelan J. 2014. Anterograde and retrograde regulation of nuclear genes encoding mitochondrial proteins during growth, development and stress. *Molecular plant*, 7: 1075-1093.
- [42.] Nuugulu LM. 2013. Growth and physiological response of amaranth seedlings to temperature and drought stress, University of Free State.
- [43.] Obidiegwu JE, Bryan CJ, Jones HG, Prashar A. 2015. Coping with drought: stress and adaptive responses in potato and perspectives for improvement. *Frontiers in plant sciences*, 6.
- [44.] Ooi MK, Denham AJ, Santana VM, Auld TD. 2014. Temperature thresholds of physically dormant seeds and plant functional responses to fire: variation among species and relative impact of climate change. *Ecology and evolution*, 4: 656-671.
- [45.] Piskurewicz U, Lopez-Molina L. 2016. Basic Techniques to Assess Seed Germination Responses to Abiotic Stress in *Arabidopsis thaliana*. *Environmental Responses in Plants: Methods and Protocols*: 183-196
- [46.] Rong Y, Li H, Johnson DA. 2015 Germination response of *Apocynum venetum* seeds to temperature and water potential. *Journal of Applied Botany Food Quality*, 88.
- [47.] Shaban M. 2013. Effect of water and temperature on seed germination and emergence as a seed hydrothermal time model. *International Journal of Advanced Biological and Biomedical Research*, 1: 1686-1691.
- [48.] Shafique A, Rehman S, Khan A, Kazi AG. 2014 Improvement of Legume Crop Production under Environmental Stresses Through Biotechnological Intervention. *Emerging Technologies and management of Crop Stress Tolerance*: 1
- [49.] Soltani E, Soltani A, Galeshi S, Ghaderi-Far F, Zeinali E. 2013. Seed bank modeling of volunteer oilseed rape: from seeds fate in the soil to seedling emergence. *Planta Daninha*, 31:267-279.
- [50.] Vashisha A, Joshi Dk. 2016. Growth characteristics of maize seeds exposed to magnetic field. *Bioelectromagnetics*.
- [51.] Wang R, Bai Y, Tanino K. 2005 Germination of winterfat (*Eurotia lanata* (Pursh) Moq) seeds at reduced water potentials: testing assumptions of hydrothermal time model. *Environmental and Experimental Botany*, 53: 49-63.
- [52.] Werle R, Sandell LD, Buhler DD, Hartzler RG, Lindquist JL. 2014. Predicting emergence of 23 summer annual weed species. *Weed science*, 62: 267-279.
- [53.] Wright A, Schnitzer SA, Reich PB. 2015. Daily environmental conditions determine the competition - facilitation balance for plant water status. *Journal of Ecology*, 103:648-656
- [54.] Yang S, Li X, Yang Y, Yin X, Yang Y. 2014. Comparing the relationship between seed germination and temperature for *Stipa* species on the Tibetan Plateau. *Botany*, 92: 895-900.
- [55.] Zaritzky N. 2015. The role of water in the Cryopreservation of seeds. *Water Stress in Biological, Chemical, Pharmaceutical and Food Systems*: Springer.p. 231-244.
- [56.] Zhang W, Wang G, Falconer JR, Baguley BC, Shaw JP, Lui J, Xu H, See E, Sun J. 2015. Strategies to maximize liposomal drug loading for a poorly water-soluble anticancer drug. *Pharmaceutical research*, 32: 1451-1461.
- [57.] Zhang YJ, Xie M, Zhang XL, Peng DL, Yu WB, Li Q, Zhao JJ, Zhang ZR. 2016. Establishment of polyethylene-glycol-mediated protoplast transformation for *Lecanicillium lecanii* and development of virulence-enhanced strains *Aphis gossypii*. *Pest management science*, 72: 1951-1958.