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Effects of high temperature on pea (*Pisum sativum*) seed quality and attributed traits

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ABSTRACT

The experiment was set up in a randomised complete block design with four replicates and two pea cultivars (Greenfeast vs. Snow pea), at two different temperature regimes (25/15-35/25°C and 25/15°C). The objective of this study is to investigate the effect of high temperature on seed quality, germination and evaluates the changes in the protein and total soluble sugar content of pea seeds in response to high temperature. The high temperature had significantly reduced flower number, flower accumulation number, pod number and pod accumulation number. The pod number of Greenfeast was highest at day 104.10 at 15/15°C than Greenfeast at 15/25-35/25 °C whereas Snow pea pod number was significantly higher at day 91.97 at 25/15 °C than at 25/15-35/25 °C and the rate of increase was higher for the Greenfeast at 25/15 °C, followed by Snow pea t 25/15-35/25 °C with the highest correlation (R^2=0.73). The pod accumulation rate (K) for Greenfeast was lower than other treatments, with maximum peak at day 98.73. The highest correlations are detected between pod accumulation rate *(k)* Snow pea at 25/15 °C and Greenfeast at 25/15-35/25 °C (R^2=0.96).

There were more Snow pea seeds germinated at 25/15 ºC in the first 5 days while the final germination rate was not significant between combination treatments ($F_{3,28}$ = 0.92, P = 0.4421). High temperature reduced Snow pea dry shoot weight at 25/15-35/25 °C compared to Greenfeast which had a low significant dry shoot weight at 25/15ºC. Root dry weight of both cultivars was reduced with high temperature (25/15-35/25 °C) compared to the low temperature (25/15 °C). In terms of protein and sugar content, Greenfeast had the highest protein content and higher sugar content than Snow pea at 25/15 °C temperature regime.

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GLOSSARY OF ABBREVIATIONS

NZ	New Zealand
MPI	Ministry for Primary Industries
SW	Seed weight
HT	High Temperature
PSN	Pod Set Number
Fe	Iron
Zn	Zinc
Ca	Calcium
UK	United Kingdom
USA	United states of America
GxE	Genotype by Environment
SMC	Seed Moisture Content (%)
FAO	Food and Agriculture Organisation of the United Nations
IPCC	International Panel on Climate Change
MAF	Ministry of Agriculture & Forestry
CO_2	Carbon dioxide
S+H	Salinity and Heat
HTT	Heat Thermal Temperature
PM	Physiological Maturity
N	Nitrogen
P	Phosphorus
K	Potassium
RCBD	Randomised Complete Block Design
ANOVA	Analysis of Variance
SAE	School of Agriculture and Environment
EC	Electrical Conductivity (uS/cm/gm)
ISF	International Seed Federation
ISTA	International Seed Testing Association
DF	Days to 50% Flowering
FL	Flower
SN	Seed Number
PH	Plant Height (cm)

CHAPTER 1

1.1 INTRODUCTION

The field pea (*Pisum sativum* L.) is a cultivated species of the genus *Pisum* family Fabaceae and is widely cultivated as a cool season crop in most temperate climates and at high elevations in tropical countries throughout the world. Sometimes field pea is known as dry pea or grain pea (Huang, 2016).

Pea is a highly nutritional crop and a cheap source of protein to supplement meat protein. It has a high protein content (23-33%) (Fikreselassie, 2012). It is also rich in amino acids, tryptophan and lysine (21-25%), complex carbohydrates, high fibre (soluble and insoluble), B vitamins, folate and mineral content, such as calcium (Ca), iron (Fe) and potassium (K). Pea also contain 86–87% total digestible nutrients and are very low in sodium and fat content (Tiwari & Singh, 2012). Pea seeds are widely used in soups, breakfast cereals, processed into pea flour, pea starch, or pea protein concentrates (Slinkard et al., 1990). It is also important to understand the nutritional composition of the cultivars as soil, climate and agronomic factors can cause differences in quality and nutritional composition (Rodrigues et al., 2012).

In New Zealand, pea is grown mostly in spring when the temperatures range between 10-16 ºC to avoid frosts during flowering. Growing at too low a temperature results in a weak crop and poor emergence that contributes to low yield and quality at harvest compared to Australia where peas can be sown as winter crop (Nguyen, 2012). The major pea growing regions in New Zealand are Canterbury (East Coast, South Island) and Hawke's Bay (East Coast, North Island) followed by other regions such as Manawatu-Whanganui and Gisborne. The Wairarapa region (South-eastern North Island) used to be the largest seed export producing area (Millner & Roskruge, 2013; White, 1987) but is not currently (2017/18) due a pea weevil incursion[1].

The pea production area in New Zealand had decreased dramatically from over 37,000 ha in 1987 to 6,273 ha in 2008 (Millner & Roskruge, 2013). According to the Food and Agriculture Organisation of the United Nations (FAO), the production area in 2016 was similar at 6,093 ha, accounting for 2% of the world production (FAOSTAT, 2018), compared to the 2017 figure, where about 62,000 metric tons was produced from 8,300 ha, fetching over $84.6 million New Zealand dollars (Horticulture NZ, 2018). This indicates that farmers are coming back slowly to growing peas again, especially after pea weevil *(Bruchus pisorum)* severely impacted the New

[1] See https://www.biosecurity.govt.nz/protection-and-response/responding/alerts/pea-weevil

Zealand pea industry by halting production in the largest pea seed export production area of the Wairarapa.

The export value from both processed and dried peas is almost $90 million dollars as reported by Statistics New Zealand (2017) while exported seeds in 2016 alone produced a monetary value of US$119 million (NZD $172 million) from 24,411 tonnes of seeds exported (ISF, 2018). Seed is the most important input for agriculture production, however if not managed well under abiotic stress conditions such as drought and high temperature seed quality will reduce which will affect global food security (ISF, 2018).

Climate change poses serious challenges to high quality seed production throughout the world and in peas, the impact of climate is that the high temperature stress effect on seed production will adversely affect production (ISF, 2018). The high temperature influences the physiological and biochemical process, thus affecting the overall plant growth and seed productivity (Todorova et al., 2016). For example, a high temperature effect on rice (*Oryza sativa),* maize (*Zea mays*) and sorghum (*Sorghum bicolor*) results in low seed germination when temperature increases to 50ºC (Iloh et al., 2014).

Field pea is very sensitive to high temperatures and seed production starts to decline when the maximum daytime temperature increases to above 25 to 30 °C (Guilioni et al.,1997; Guilioni et al., 2003; Pumphrey & Ramig, 1990). In addition, when the temperature is over 35ºC, it is considered more severe for pea seed production (Munier-Jolain et al., 2010). The high temperature effects at flowering stage, anthesis, growth and development, and grain-filling stages have been extensively studied in maize (*Zea mays*), sorghum (*Sorghum biocolar)*, common beans (*Phaseolus vulgaris),* cowpeas (*Vigna unguiculata*) (Luo, 2011), rice *(Oryza sativa)* (Chaturvedi et al., 2017) and wheat (*Triticum aestivum* L.) (Gupta et al., 2015) using phenological, morphological and physiological approaches. High temperature (25-35°C) and water deficit was also cited to be the cause of hollow heart disorder in peas after onset of pod wrinkling (Halligan, 1986; Heydecker & Kohistani, 1969). Pea seed production has been extensively studied in New Zealand including plant production, pest and diseases, irrigation requirements, fertilizer (Wilson, 1987) nutrition, time of sowing, and harvesting methods on seed quality and yield (Padrit, 1996), population density, production factors and seed quality characters under field conditions (Castillo et al.,1993a; 1993b), sowing, growth and development (Nguyen, 2012). However, impact of increased temperature on pea seed production has not been studied with little information available to date. Pea breeding in New

Zealand is undertaken by Plant and Food Research (PFR) located in Christchurch, South Island and Canterbury regions. Efforts to improve pea seed yield and quality in New Zealand focussed on enhancement of seed yield and quality with resistance to powdery and downy mildew (A. Russell, personnel communication, September 1st, 2017). Screening for resistance to extreme temperatures in cool season food legumes like pea is complicated by lack of adequate screening techniques, environments and lack of selection criteria. Developing new screening methods requires better knowledge of adaptive traits to heat tolerance, salinity tolerance and drought as these events occur in combination and sometimes is difficult as these traits are polygenic and makes the prediction of yield under such condition unreliable (Huang, 2016). Evaluating through Genotype by environment interactions (GxE) are common under abiotic stress making breeding and evaluation progress difficult (Banziger et al., 2006).

New Zealand has a temperate environment, therefore the effect of temperature and drought on pea yield is not considered important unlike in Australia. However, it is considered important during summer months. This study was carried out to determine the effect of high temperature during pea seed production on pea seed quality. Thus, the specific objectives of this study were to:

- Study the effect of high temperature on seed quality, germination and associated traits
- Evaluate the protein and soluble sugar content of pea seeds in respond to high temperatures

1.2 LITERATURE REVIEW

The following review addresses the effect of high temperature on pea and similar legumes. The areas covered are field pea and its production, temperature stress effects on New Zealand agriculture, seed vigour which discusses hollow heart and conductivity, high temperature stress on seed germination, threshold temperature, high temperature effects on vegetative growth and reproductive development. This is followed by adaptation mechanisms for high temperature stress, and breeding strategies which cover conventional plant breeding. The quality traits refer to protein, sugars, and the effects of high temperature on the grain composition and quality.

1.3 PEA (*PISUM SATIVUM* L.)

The pea (*Pisum sativum* L.) is among the over 18,000 species recognised in the Leguminosae (Fabaceae) family. Also included are the genera *Lathyrus* (160), *Lens* (4) and *Vicia* (160-250) species (Mikic et al., 2013; Schaefer et al., 2012; Smykal et al., 2011). The *Pisum lanthyrus* taxa has changed over time (Kupicha, 1981 as cited in Ron, 2015), from a genus of five to two species, *Pisum sativum* and *P. fulvum,* but there are other wild relative species including (*P. elatius*, *P. abyssinicum*, *P. sativum, P. humile P. syriacum, P. sativum* subsp. *sativum* var. *Pumilio)* recorded in a review by Yarnell (1962). The breeding of interspecific pea hybrids is complicated due to cross-incompatibility. A crossing experiment was undertaken between *Lathyrus* and *Pisum* species and was shown to have cross-incompatibility problems (Smykal et al., 2011).

The origin of the pea (*Pisum sativum*) is obscure, although it is one of the world's oldest domesticated crops dating back to 1800 BC (Rana et al., 2017). It was cultivated along with barley and wheat almost 7000 years ago (Jaskanwal, 2011).

According to Vavilov (1926), pea was discovered near the Mediterranean and the near East, Ethiopia and Central Asia, but it has now been widely distributed by humans to many temperate parts of the world, including Netherlands, France, UK, USA, Australia, and New Zealand (Riehl et al., 2013). The pea was among other major grain legumes that accompanied cereals to form the dietary components of early civilization in the Middle East and across the Mediterranean. The pea has been cultivated in Europe since the stone and bronze ages, in India since 200BC (De Candolle, 1885; Jaskanwal, 2011) and New Zealand since the 1900's (Jermyn, 1987).

Among the different pulse crops such as the common bean, soybean *(Glycine max*), chickpea *(Cicer arietinum)* and faba bean *(Vicia faba)*, the pea (*Pisum sativum)* is classified into processed and garden or field peas. The processed peas are sometimes referred to as green peas or vegetable peas, and are mainly consumed as fresh pods, fresh seed or as canned vegetables while the field peas or garden peas or dry peas are marketed as dry and shelled for both human food and livestock consumption.

The field pea or garden pea is an important nutritional seed crop. They are very low in fat, high in complex carbohydrates, including soluble and insoluble fibre (Sharma et al., 2015; Huang, 2016) and are recognised globally as an important source of protein, vitamins and minerals (Ma et al., 2017). Research in Europe has demonstrated that eating legumes like pea and other legumes allows people to live longer, and prevents diabetes, cardiovascular disease and cancer

(Huang, 2016; Olle, 2017; Sharma et al., 2015; Weigel, VanRaden, Norman, & Grosu, 2017). Several other researchers have reported that peas are an excellent protein supplement in pig, dairy and poultry (Huang, 2016; Rodrigues et al., 2012) Therefore, the pea has important nutritional benefits for animals and humans. However, the impact of higher temperatures on peas can reduce pea seed production and affects some of its quality contributing to reduction in nutritional benefits (Shukla, 2015).

1.3.1 Pea production

Being the model crop of choice in Mendel's discovery of the laws of inheritance, the domestication of the field pea can be traced back to 1800 BC. Peas were cultivated along with barley and wheat almost 7000 years ago (Jaskanwal, 2011; Rana et al., 2017). Despite many studies to trace the origin of the crop, it was not until 1926 that Vavilov (1926) and other researchers confirmed that the pea had originated near the Mediterranean, Ethiopia and Central Asia. The annual production area for peas in the world is five million hectares which produce about seventeen million tonnes annually, which ranks the crop as the third most important grain legume after the common bean and soybean (FAOSTAT, 2017). The top five producing countries are Canada, India, China, France, and Russia based on the 2018 production figures (FAOSTAT, 2017 & 2018).

In New Zealand, pea has been cultivated since the 1900s with the seeds having been brought in by British explorers since 1769. The main cultivars were from England known as , splitting peas and maple peas produced for export to Europe (Jermyn, 1987). Research into the pea seeds for production started in 1928 at the Government experimental farm in Ashburton and later continued at the Plant and Research Bureau, now known as Plant and Food Research Ltd, a Crown (Government) Research Institution. The early pea breeding programme commenced in 1932 (Jermyn, 1987; Thieme, 2008). The breeding objectives were targeted at breeding for resistance to disease, followed by quality and yield.

The breeding program contributed to the development of a number of varieties that undergo extensive field trials 1937 to 1941. For example blue Prussian, however, it did not meet other criteria such as hardiness, colour and water absorption, apart from yield and quality (Jermyn, 1987) and was not promoted for cultivation.

From these modest beginnings, according to the most recent data by FAOSTAT, the harvested area is now 12,000 ha, producing over six hundred thousand metric tons with the yield production of 99,603 metric tons in both dry and green pea beans (FAOSTAT, 2018). The

number of pea growers in 1999 was 900. This has declined dramatically over the past decade to 400 in 2015, but for 2016-2017, the growers' numbers increased to 450 farmers. The annual export of dry peas is 62,000 metric tons earning about $50 million from domestic sales and $84.6 million from exports as dry frozen packages annually to New Zealand's revenue (New Zealand Horticulture, 2018).

The major pea growing area in New Zealand is Canterbury with 3,169ha (2017 figures), followed by Hawke's Bay with 2,126ha (2017) of cultivated land. The area and grower numbers are slowly increasing. However, some growers are moving into growing crops like maize, barley and oats that provide a higher profit margin (Horticulture New Zealand, 2018). On the other hand, production can be affected by factors such as disease, pests, and high temperature caused by climate change, contributing to decline in yield. The recent pea weevil threat in the Wairarapa region is an attestation to the grower movement to alternative crops.

1.4 TEMPERATURE STRESS

1.4.1 Impact of temperature on the New Zealand Agriculture

The arable crops globally account for 50% of the calories consumed by humans. This is from cereal crops like maize, rice and wheat (Clark et al., 2012).The global demand for maize, wheat and rice accounts for 90% of the world's cereal production (Stewart & Lal, 2018). The world population is projected to increase to 9 billion by 2050, thus food production would need to increase by 70% to support this population (New Zealand Agricultural Greenhouse Gas Research Centre (NZAGGRC), 2012; Pataczek, 2018; Stewart, 2018). The global mean temperature is reported to increase by 1.45 to 4.5 ºC by 2050 (Houghton et al., 2001). These increases are due to the increase in CO_2 concentrations combined with greenhouse gasses in the atmosphere caused by anthropogenic activities (IPCC, 2007).

The increase in climate change impacts such as increase in temperature will impose additional challenges for the New Zealand arable agriculture industry. New Zealand arable crops such as wheat, barley, maize and oats covers about 165,000 ha, whereas vegetables such as potatoes, onions, carrots and peas cover 55,000 ha while the remaining areas in arable production are devoted to forage crops making up 350, 000 ha (Teixeira et al, 2012a). It is projected that for New Zealand, the temperature will increase from 1 to 4 ºC by 2090 (Teixeira et al., 2012). The increase in atmospheric carbon dioxide (CO_2) concentration, increase in temperature, and change in amount of precipitation and its distribution will affect the performance of these crops, including vegetable crops such as peas for both seed and yield production. The high

temperatures will influence crop development rates by shortening the growth period, shortening the timing of flowering, canopy expansion, respiration, and transpiration. Additionally, the increase in temperature will also contribute to an extended growing season by allowing earlier planting and later harvesting (Teixeira & Brown, 2012) and, more significantly, will reduce yield. According to Zhao et al. (2017), high temperatures will reduce global yields of major crops such as wheat (6%), rice (3.2%), maize (7.4%) and soybeans by (3.1%). Furthermore, studies have shown that high temperatures lead to premature seed head development in some crops or delayed curd initiation or increased leaf numbers as in cauliflower. Higher temperatures over the last couple of decades have encouraged the spread of kikuyu (*Pennisetum clandestinum*) and paspalum, (*Paspalum dilatatum*) sub-tropical pasture grasses in the North Island of New Zealand, which are considered detrimental to dairy production (Kenny, 2001; Ministry of Agriculture and Forestry, 2009). Additionally, changes in temperature could also lead to an increase in pests and disease. For example, in Northland, insects such as tropical armyworm (*Spodoptera litura*), guava moth (*Coscinoptycha improbana*), and in Hawke's Bay clover weevil (*Sitona lepidus*), clover flea (*Sminthurus viridis*) and crickets (*Gryllidae* family) population are increasing with changes in climatic conditions (Ministry of Agriculture and Forestry (NZ), 2009)

Despite the negative side effects, the increased temperature effects will be offset by the fertilising effects of carbon dioxide concentration increasing the yield of temperate crops like wheat and barley, and crops such as maize. These crops utilize CO_2 differently from other temperate crops that will show no improved yields in response to high CO_2. The high temperature provides an opportunity for growing maize with other cooler crops including peas to increase production. It is anticipated that an increase in carbon dioxide will increase crop production to 19 percent by 2040 and 37 percent by 2090 (Watt et al., 2018). It is reported that onions, beetroot, carrots have increased in yield, while French beans showed no yield response to high CO_2 levels (Brent et al., 2012).

New Zealand temperatures are projected to increase from 1 ºC by 2040 and 2 ºC by 2090 (Beresford & Mackay, 2012; Teixeira & Brown, 2012). With these changes, the New Zealand climate is likely to become more sub-tropical especially in the north, drier in the east with a milder, more temperate climate developing in cooler and southern regions, and windier and wetter in the west of the country which will have a positive effect on the pea production in the Southern regions than the Northern regions of the country (New Zealand Agricultural Greenhouse Gas Research Centre (NZAGGRC), 2012). This will influence the yield due to the

increase in carbon dioxide concentrates, increases in seasonal temperatures and shifts in rainfall patterns will also affect seed quality. The North Island is most vulnerable, in particular, to hotter summers and stronger seasonal rainfall including the major pea seeds growing area of the Hawke's Bay and Wairarapa regions.

The effect on rainfall is more variable in many western regions of the country. The rainfall is predicted to increase slightly (0-2.5%) by 2040, but in eastern regions including Canterbury, rainfall is expected to decline (0 to 2.5%) (Teixeira & Brown, 2012) creating a drier conditions increasing the temperatures which could have a detrimental effect on the reproductive development and growth of pea which could reduce potential seed yield. Peas are going to be affected where the mineral concentration in plants will be reduced affecting the seed development in the pods (Hampton et al., 2013; Kohler et al., 2019).

1.5 SEED VIGOUR

Seed vigour is defined as the sum total of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence''. Seed vigour test is an important quality indicating the quality and value of the seed lot than the standard seed germination test (Khan et al, 2010 & Gupta et al. 2015) either in the field or in storage and is measured by electrical conductivity. Furthermore, an electrical conductivity test is a measure of the electrolytes leaking from the plant tissue. The test was first conducted on cotton in 1958 as reported by Padrit (1996).

The test is now widely accepted in many groups and used widely in Australia, New Zealand and the rest of the world. The electrical conductivity (EC) test for garden peas is one of only two vigour tests included in the ISTA Rules for Seed Testing (ISTA, 2006), though it existed for a long time (Matthews & Powell, 2006). The test is useful for many legume crops such as field bean, soybean and French bean (Padrit, 1996).

1.5.1 Hollow heart

Hollow heart is a physiological disorder which was discovered and named by (Myers, 1948). Hollow heart appears on the adaxial surface of the cotyledons in germinating pea seeds and are characterised by a concave depression on the cotyledons (Heydecker & Kohistani, 1969; Perry & Harrison, 1973; Perry & Howell, 1965). The depression can vary from shallow to deep with cracks across the middle of the cotyledons and can cause emergence failure under field conditions

Several studies were carried out on different seed lots, from wrinkled to smooth-coated seed lots. Heydecker and Kohistani (1969), found that seed lots from wrinkled seed Kelvendon Wonder variety were free from hollow heart, while those of Gregory surprise, a smooth-coated variety, showed a high incidence of hollow heart in germination testing as well as poor field establishment in Scottish field trials, suggesting that hollow heart incidence will vary across varieties. Hollow heart is reported to be common in all garden pea seed lots in New Zealand, (Shinohara et al., 2006a). The disorder predisposes seedlings to pathogenic fungal attachment, delayed germination, reduced emergences and seedling growth resulting in yield reduction (Perry & Harrison, 1973). Castillo et al. (1993a) found that hollow heart contributes to decreasing field pea emergence under wet soil conditions which are common in early spring in New Zealand. Some of the causes of disorder have been suggested by (Perry & Harrison, 1973) which include oxygen deficiency during seed imbibition, physical stress during maturation, and drying seeds rapidly (Allen, 1961; Perry & Howell, 1965). Hollow heart is also associated with high temperatures (Shinohara et al., 2006b). The effect of drying seeds at high temperatures were investigated in a glasshouse experiment where all the pods were harvested after 12 weeks of sowing (Perry & Harrison, 1973). They found that the incidence of hollow heart increased when the drying temperature increased but decreased as seed maturity was extended. They also found that the effect of air temperature during seed ripening was not correlated with maximum, minimum and average daily temperatures for periods longer than ten days.

1.5.2 Temperature effects on seed germination

Successful crop production depends on the seed quality, rate of germination, seed mass and seed vigour. According to Huang (2016) stated the optimum temperature to encourage germination and growth development in pea is 13-20 °C and 15-25 °C respectively which is also accepted as the threshold for pea germination and growth and is in agreement with (Sita et al., 2017).

When the air temperature is above the optimum level, it can affect vegetative development by reducing germination percentage, seedling emergence, increase abnormal seedlings, poor seedling vigour and reduced radical and plumule development in legumes (Sita et al., 2017). The temperature in which seeds are able to germinate also depends on crops species, for example, maize (*Zea mays*) performs best at 10-40 °C, wheat (*Triticum aestivum* L) at 20-40 °C, soybean at 10-35 °C, peas at 15-25 ºC (Huang, 2016; Sita et al., 2017) and lettuce (*Lactuca*

sativa) and spinach (*Spinacea Oleracea*) range from 15-20 ºC (Khalil et al., 2018). However, a high temperature also has detrimental effects on germination. Reduced seed germination at a high temperature has been reported in many legumes including soybean, pea lentil, mung bean and chickpea by various authors (Sita et al., 2017). A study on the effect of higher temperatures in soybean, and common bean reveals that exposure to 28°C for eight days resulted in 50.4 and 36.2% seedling germination in non-irrigated soybeans and common beans. Likewise, seed germination and vigour index in mung bean (*Vigna radiata*) exposed to 50 °C for 10, 20 and 30 minutes showed a significant reduction (Nandagopalan, 2017; Sita et al., 2017). In addition, the lentil seeds exposed to 35-40 ºC for 4 hours reduced germination and seedlings showed retarded growth (Chakraborty, 2011).

The germination of a seed lot can be affected by the parental environment that can negatively affect the seed quality. This includes the unfavourable growing conditions in the field during growth and development of the seed on the parent plant, harvesting, drying of the seeds, cleaning and storage. Factors such as temperature and relative humidity can affect seed development and maturation under field conditions. High temperatures during the seed filling can disrupt normal seed development, increase the proportion of shrivelled seeds, and produce abnormal and lower quality seeds (Hampton et al., 2013, Fenner, 1991).

High temperature stress before the developing seeds achieve physiological maturity (PM) is likely to inhibit the ability of the plant to supply the seeds with the essential storage compounds required during germination.

In soybean, germination and seed vigour were reduced when the ambient temperature increased (Dornbos & Mullen, 1991). The effect of seedling vigour and viability was studied in pea and soybean (*Glycine max*) (Anto & Jayaram, 2010). They revealed that high temperature treatment reduced the germination percentage and moisture content, and seed and seedling vigour. The high temperature effects on peas were investigated where the plants were exposed to a day / night temperature of (30/20 ºC) for four days above the base temperature (TB) for 25 ºC at the beginning of seed filling until seed harvest; germination was lower in the cultivar Alderman compared with Onward, suggesting cultivar effects in the response to temperature. Exposure to higher temperatures at the later stages of seed development did not affect seed germination (Shinohara et al., 2006a).

Heat stress during seed development in *Brassica* and the effect on seed quality was investigated for two consecutive seasons on forage rape (*Brassica napus*) in New Zealand. The plants were

exposed to 30/25 ºC heat stress over a 240°Ch. The result showed significant reduction in germination in both seasons due to abnormal seedling development and also reduced seed vigour and seed mass (Rashid et al., 2018). The effect of high temperatures on two hybrid rice cultivars was that seed set was reduced by high temperatures in the three cultivars studied (Madan et al., 2012).and that the yield was affected by a temperature of 38 °C but a high yield was produced at 29 ºC. The elevated effect of temperature on seed development and maturity can adversely affect quality contributing to low production.

The seed mass refers to seed size which refers to seed volume, whereas seed weight and seed mass refers to density which are different traits (Hampton et al., 2013). Water availability and nutrient availability are two genetic factors which affect seed mass.

An increase in temperature may reduce seed mass (Spears et al., 1997) due to the acceleration in seed growth rate (dry matter accumulation) and reduction in the duration of seed filling. However, a reduction in the seed dry matter accumulation can also occur in a seed mass; sometimes no change has been reported and sometimes there has been an increase with an increase in temperature (Peltonen-Sainio et al., 2011). The reduction in seed mass for seed lot does not mean that other seed quality attributes are affected. Some studies showed no relationship between the seed mass and seed germination (Hampton et al., 2013).

Seed vigour is defined, as the sum of those properties that determine the activity and level of performance of seed lots of acceptable germination in a wide range of environments (ISTA, 2018). High temperatures reduce seed vigour before physiological maturity (PM) and after PM (Egli et al., 2005; Shinohara, 2006; Spears, 1997). A vigour test was conducted for 262 garden pea seed lots produced in New Zealand and the climate data for five major regions over four consecutive production seasons by Shinohara et al. (2008). It was found that these seasonal variations were significantly associated with high temperatures during seed development.

High temperatures also reduced vigour in the growth chamber in phytotron experiments (Egli et al., 2005). Temperatures of 33/28 ºC day/ night (Spears, 1997), 38/27 ºC (Egli et al., 2005) during seed filling, reduced germination from several cultivars. Seed vigour was reported to be more sensitive to high temperatures than standard germination. In soybean, seeds exposed to high temperatures during the seed filling stage were shrivelled or more abnormal than seeds with no impaction. The variety Hutchson showed more reduction in seed vigour than DP4690 was surprising as this variety is mostly grown in mid-south regions of the USA where summer temperatures are very high (Egli et al., 2005).

The seed vigour loss due to high temperatures also depends on the stage of the development. (Halligan, 1986; Shinohara et al., 2006a) found in field pea studies that the thermal time (HTT) measured in degree hours °Ch, (Tb =25 °C) when seeds were at the wrinkled pod stage (700-800mg/g) seed moisture content (SMC) was highly correlated with hollow heart incidence at harvest. A confirmation experiment was carried out in the control environment by Shinohara et al. (2006b) where the peas were exposed to a day and night temperature (30/25 ºC day/night) for four days (240ºCh, Tb=25 ºC). At the wrinkled pod stage it induced hollowed heart, but exposure to the same temperatures at the beginning of seed filling (>800mg/g SMC), PM 550-650 mg/g SMC) after PM did not show hollow heart. Rashid et al. (2018), studied the effects of heat stress on seed development in *Brassica napus* L). The plants were exposed to heat stress of 30/25°C during the seed filling stage. The results show that germination, plant vigour and seed mass were all reduced with high production of abnormal seedlings.

1.5.3 Temperature stress and threshold temperature

Temperature is a major factor affecting seed yield and quality in legume crops. The increase in air temperature by one degree above the threshold can induce detrimental effects on plant growth development, seed and yield. In most of the sub-tropical and tropical crops, the temperature threshold is 32-25 °C (Sita et al., 2017). However, in the cool season crops the maximum threshold is 25 ºC (Wahid et al., 2007). The effect of temperature stress depends on intensity, duration of the exposure and the degree of the elevated temperature (Davisirvatham et al., 2012, Sita et al., 2017). According to Wahid, Gelani, Ashraf, and Foolad (2007) extreme temperatures, both high and low, can have adverse effects on plant growth by weakening plant growth and development functions. The severity of variation of high temperature effects on crops varies from region to region and year to year making it difficult for selection of suitable stress tolerance cultivars. Huang (2016) classified the severity of the heat stress into two groups based on timing and duration. These two groups are chronic and acute. Chronic heat stress refers to long-time range of mild stress where temperature is some degrees above optimal, unlike acute stress which is a shorter period of stress, but with high temperature and higher than few degrees. Temperature stress can impose challenges in plants at various stages with severe impacts on the vegetative and reproductive growth (Hamidou et al., 2013). It can also disrupt the physiological processes of plants resulting in reduced nitrogen anabolism, photosynthetic

inhibition as well as high protein catabolism, an accumulation of end production of lipids as reported by other researchers (Sita et al., 2017).

Cool-season food legumes are more sensitive to high temperature stress than warm-season food legumes and the ideal temperature for plant growth is 10-30 ºC. The critical temperature for temperature tolerance seems to be higher in chickpea (15-30 °C), and lentil (15-30 ºC), than in faba bean (18-28 ºC), and field pea (15-25 ºC) degrees, and the reverse is true for cold tolerance (Sita et al., 2017). This is consistent with those reported by Huang (2016) that the chickpea performs best at 20-29 ºC air temperature during the day, lentils grow best at 15-27 ºC, followed by common bean 16-26 ºC and pea grow best at 13-23 ºC. In rice, the best temperature for vegetative growth is 33 ºC and for wheat 20-30 ºC which are different from reproductive yield phase temperatures which are 23-26 °C for rice and 15 ºC for wheat respectively (Hatfield et al., 2008; Huang, 2016). Generally, a maximum temperature of 25 °C is regarded as the threshold level for temperature stress in most of the crops, but any temperature above this threshold can affect vegetative structures in plants. Plants grown under high heat or temperature stress show shorter vegetative and pod-filling periods (Adams et al., 2001), poor crop stand, consequently reduced yield.

1.5.4 Effect of temperature stress on vegetative growth performance of peas

Additionally, vegetative parts of a plant respond to high temperature stress in various ways by showing different symptoms on the plant parts. Some of these common responses are scorching, sun burning of leaves, twigs, branches and stems, senescence of leaves, followed by abscissions, imbibition of shoot and root growth, and discoloration of fruits which can severely reduce yield. Furthermore, exposure of plants to high temperatures reduce shoot growth, root number, root diameter, cause leaf wilting, leaf curling, leaf yellowing and reduce plant height and biomass (Siddiqui et al., 2015). All these reduce the value of the plant as a source of nutrients for the developing seed.

High temperatures are reported to have severe effects on vegetative growth in some of the common legumes such as peanut (at 29-33 ºC), peas (at 28-30 ºC), and chickpea (at 22-25 ºC) (Siddiqui et al., 2015; Sita et al., 2017). High temperature also reduces cell size and growth, thus reduces leaf area and biomass. Plant height is reduced due to the decline in stem growth under high temperatures, and for weight, it has been reported that the size of the leaves are all reduced due to reduced cell elongation rate (Khalil et al., 2018). An experiment was carried out to investigate different faba bean growth responses to heat stress. The results showed that plant

height, shoot fresh weight, dry weight, and leaf area decreased under high temperatures compared with the control treatment (Siddiqui et al., 2015). Exposure to high temperature (30/25 ºC) induced early senescence of the lower leaves of peas (Huang, 2016). As highlighted by McDonald and Paulsen (1997), high temperatures decrease chlorophyll variable inflorescence (Fv), a measure of injury to photosynthesis in five pea cultivars. They also found thylakoid activity was reduced when heating was increased to 40 °C for 2.5 minutes. Similarly, Kumar et al., (2013) reported that stomatal conductance, leaf water content, chlorophyll, membrane integrity and photochemical efficiency declined in heat sensitive genotypes. High temperature effects on membranes showed an increase in fluidity which is detected by membrane senses. High temperatures decreased the physiological performance and growth of two warm season grasses *Andropogon gerardi* (a C4 grass) and *Solidago canadensis* (a C3 grass) under prairie vegetation (Wang et al., 2016). High temperature also reduce root size, length and number, relative growth rate, dry weight of shoots and net assimilation rate in millet (Ashraf & Hafeez, 2004). Therefore, high temperature can inhibit vegetative growth performance.

1.5.5 Effect of temperature stress on reproductive development

The high temperature effects on reproductive organ development has been widely reported in many legumes including common beans (Gross & Kigel, 1994), chickpeas (Kaushal et al., 2013; Kumar et al., 2013), and peas (Guilioni, 1997), whilst in cereal crops it was reported in barley, maize, rice and wheat (Sita, 2017; Wahid, 2007). The reproductive organs starting from flower initiation through to seed development respond differently to high temperature stress (Sita et al., 2017). High temperature is lethal to flower buds and fruits, pods and seeds (Kaushal et al., 2016). High temperatures are reported to reduce flower numbers and branches. At high temperatures male and female gametophytes are disrupted contributing to poor pollen viability, poor pollen germination, restriction of pollen tube growth, failure of stigma receptivity and ovule function, decrease of ovule viability, limit embryogenesis, increase of ovule abortion and poor seed set (Gupta et al., 2015; Kumar et al., 2013; Sita et al., 2017).

During flowering formation and development, a mild or high temperature above 30 ºC is considered detrimental to both male and female flowers (Lavania et al., 2015) thus has severe consequences on flower bud initiation and can take 10-15 days (Bita & Gerats, 2013) as shown in a study on faba beans (Bishop et al., 2016, Lavania et al., 2015). High temperatures can also decrease the number and size of flowers, deformation of floral organs resulting in loss of

flowers and young pods, hence a reduction in seed yield in chickpeas and mung beans (Sita et al., 2017). In another study, high temperatures during the flowering period reduced pod formation, seed set in chickpeas (Devasirvatham et al., 2013), and reduced the yield in faba beans (Bishop et al., 2016). High temperatures affect anthesis, pollen fertility, pollination, female fertility, early zygote development, and seed yield. Anthesis is affected by initiating the reproductive stage prior to accumulation of sufficient resource (Bita & Gerats, 2013). High temperatures result in lower seed set due to male sterility in most legumes including field peas (R. Zhou, Wu, Cao, & Jiang, 2015). In most legumes, male gametes are more sensitive to high temperatures than female gametes. High temperatures encourage early abortion of tapetal cells which leads to pollen sterility, inhibits style length and consequently induces abnormality in ovary development as observed in chickpeas. Temperatures above 40/30 °C and 45/35 °C reduce stigma receptivity and pollen germination, stigma and style growth (Kumar et al., 2013) and reduce ovule numbers and viability at 30 °C. For example, in the common bean, these temperature highs inhibit the development of fertilization by male and female gametes, reduce fertilization efficiency due to high stress. They also reduce viability of pollen grains when the temperature is above 30ºC in chickpeas resulting in fertilization failure. Further abnormalities in anthers and pollen have been reported in the common bean (*Phaseolus vulgaris*) (32/27 ºC) and cowpeas (*Vigna unguiculata*) (33/30 ºC) (Khalil et al., 2018; Sita et al., 2017)

Pollen production is important for the fertilization process. At high temperatures 30-80 % pollen reduction has been reported in soybeans due to poor pollen tube growth, poor pollen germination and poor fertilization of the ovule (Koti et al., 2004). In another study by Jiang, (2018), pea showed a negative relationship in the number of flowers, viable pollen, pollen tube length and pollen germination and this was further confirmed by Zhou (2018), when they studied the plant at anthesis at 36/18 ºC in a growth chamber. According to (Guilioni, Wéry, & Lecoeur, 2003) seed number in peas was decreased by a mild stress condition. Low pollen availability was observed in peaches and beans contributing to low fruit set (Khalil et al., 2018). Reduced pollen grains and viability was reported in chickpeas when the temperature increased above 30ºC, resulting in fertilization failure and low seed set (Kaushal, Bhandari, Siddique, & Nayyar, 2016). High temperatures of 27/32 °C also reduced pollination in heat sensitive genotypes in beans, due to pollen sterility, failed anther dehiscence causing low pod and seed set (Gross, 1994, Zhang, 2016). Adverse temperature effects on pollen viability, seed set, seed yield and harvest index of grain sorghum (*Sorghum bicolor (*L) Moench) were also investigated. The results showed that panicle emergence was inhibited at the growth temperature of

40/30 °C and 44/34 °C respectively (Prasad et al., 2016). They also showed that the growth temperature >36/26 ºC significantly reduced pollen production, pollen viability, seed-set, seed yield and harvest index when compared with 32/22 ºC. In another study, the effect of high temperature (heat stress) was carried out on two pea lines, CDC Golden and CDC Sage, ranging from 24 to 36 °C to study the effects on seed set, pollen morphology and pollen surface composition (Jiang et al., 2015). They found that high temperatures reduce the percentage of pollen germination by 30% in CDC gold and 55% in CDC golden, pollen tube length, pod length, number of seeds per pod and the seed ovule ratio when the temperature was increased from 24 to 36 °C, but pod length remained the same when the temperature was increased from 24 to 33 °C.

Some crops are sensitive to night temperatures. For example, rice spikelet fertility is sensitive to night temperature (Martínez-Eixarch & Ellis, 2015). Martínez-Eixarch and Ellis observed that spikelet fertility was reduced before anthesis under 38/34 ºC. In another experiment on two strawberries (*Fragaria spp),* a reduced number of flowers, fruits and a shorter time of ripening were reported when the temperature was increased 25 to 30 °C for fruit production (Khalil et al., 2018). The reproductive development depends on day and night temperatures and a slight change in these temperatures can drastically reduce plant reproductive development.

1.6 ADAPTATION MECHANISM TO TEMPERATURE STRESS

Plants have developed various short and long-term adaptive mechanism strategies to cope with high temperature stress by adjusting some of their life cycle to prevent high temperature effects during their active growing season. These adaptive mechanisms help them to respond accordingly to the high temperature stress situations experienced. The main mechanisms are escape, avoidance and tolerance.

1.6.1 Escape mechanism:

Plants escape high temperatures by accelerating their growth to complete a full life cycle prior to an oncoming high temperature stress or heat stress event. This strategy is widely used in native plant species (Shavrukov et al., 2017). The plant response to this mechanism is species–specific and most do this by flowering early with a shorter vegetative growth phase. Early flowering has been observed in wheat (Shavrukov et al., 2017), chickpeas (Devasrvatham et al., 2012), pea (Huang, 2016) to avoid heat stress that occurs at the end of the growing season. This strategy is commonly observed in wheat breeding and chickpeas, and peas in the

Mediterranean type climate where drought is common causing high heat stress. In breeding programs conducted in dry areas that have recommended and released cultivars, it shows varieties that escape by early flowering and maturing, have proven to be a stress escape mechanism and serve as a useful criteria to select for heat-resistant cultivars (Huang, 2016; Shavrukov et al., 2017).

1.6.2 Avoidance mechanism:

The avoidance mechanism can be achieved in plants by changing leaf orientation, transpiration cooling, alteration of membrane lipid composition, closure of stomata and reduce water loss, increased stomatal and trachomatous densities, large xylem vessels are some of the common avoidance strategies in plants (Hasanuzzaman et al., 2013, Zhang, Carlucci, Nguyen, Hayes-Jackson, & Tonsor, 2016) This mechanism is only suitable for moderate environments and not useful for stressful environments. Additionally, early maturation is reported to be correlated with small yields, and such plants achieve this by completing reproductive cycles during the cooler months prior to the heat stress (Fitter, 2012; Hasanuzzaman, 2013). Plants growing in Mediterranean environments where the climate is hot, avoid heat stress by developing a thick coating around the leaf surface and cuticle with small hairs (tomentoes) to reduce solar radiation by having the leaf blade orientation parallel to the sun's rays (paraheliotropism). Under high temperatures, plants also respond by having their leaf rolling. This characteristic is important to maintain the efficiency of water metabolism, for example, in the flag leaves of wheat (Sarieva et al., 2010).

1.6.3 Tolerance mechanisms:

This is defined as the ability of the plant to grow and produce an economic yield, and repair damaged cells while under high temperatures (Puijalon et al., 2011; Zhang et al., 2016).

The mechanism of heat tolerance has been linked to increased tolerance of photosynthetic apparatus (Asthir, 2015).The heat tolerance mechanisms include protection and repair of damaged cells structures and structural proteins and enzymes (Shah et al., 2011). Some of the major tolerance mechanisms include ion transporters, late embryogenesis abundant (LEA) proteins, osmoprotectants, and antioxidant defence, and factors that are involved in signalling cascades and transcriptional control are important to counteract the stress effects (Rodriguez et al., 2005, Hasanuzzaman, Nahar, & Fujita, 2013). However, these tolerance mechanisms are expensive metabolically. They are found in lower productivity situations such as survival and can cause a reduced yield.

1.6.4 Management Strategies for Temperature Stress

Furthermore, high temperatures can also be avoided by proper management practice such as sowing methods, cultivar types, sowing date etc. For instance, in sub-tropical zones, lettuce which is mostly sown in the summer, may show incomplete germination and emergence, but this can be overcome by sowing lettuce (*Lactuca sativa)* into the dug beds during the day and using sprinkler irrigation to water plants in the afternoons (Hasanuzzaman et al.,2013).

1.7 BREEDING STRATEGIES FOR HIGHER TEMPERATURE STRESS

The continued increasing temperature and evapotranspiration rates events are expected to increase due to global climate change. This will have a huge impact on the food security. Therefore, food security must increase, and this will depend on the release of new cultivars with good seed quality, yield and better agronomic traits with improved adaptation to high temperature stress or heat stress conditions (Rauf et al., 2016). This requires a concerted effort by breeders combined with access to enough diverse genetic resources from both landrace and wild cultivars to form the base population that exhibit traits that are resistant to disease and pests, including environmental stress such as temperature stress, from which selections can be made. The effect of high temperature effects during the reproductive phase of plants are widely reported on pollen tube growth in rice (Rao et al., 2016) pea (Devasirvatham et al., 2012, Jiang et al., 2015), cowpeas, common beans and soybeans (Huang, 2016).

However, traits related to high temperature or heat stress or drought are polygenic or complex and are dependent on the environment and the genotype interactions, which makes selection difficult. The assessment and selection of better traits for such conditions can now be done through genomic and marker assisted selection through introgression of targeted traits from new lines and genes that are controlling the trait. Marker assisted selection can accelerate the screening and shorten the breeding process. If reliable markers are developed for the crop of interest (Huang, 2016, Rauf, 2016).

1.7.1 Conventional Plant Breeding

Plant breeding is simply the process of crossing the best parent species, identifying and recovering the best performing progeny over the parents. This process is developed following the Mendals Law of Genetic Inheritance (1900) and the natural selection concept by Darwin (1859) (Hallauer, 2011). The process of plant breeding involves germplasm collections,

identification of superior phenotypes and their hybridization to develop improved varieties (Fehr, 1897). However, screening for resistance to extreme temperatures and moisture in cool season food legumes like peas is complicated by lack of adequate screening techniques, suitable screening environments and lack of selection criteria. Developing new screening methods requires better knowledge of adaptive traits to heat tolerance and drought as these events occur in combination. Genotype by environment interactions (GxE) are common under abiotic stress making breeding and evaluation progress difficult (Bänziger et al., 2006) because heat stress is usually accompanied by drought, salinity and other abiotic stress that sometimes makes the prediction of the yield trials under such conditions unreliable (Huang, 2016). Study of tomato *(Solanum lycopersicon)* demonstrated that plants grown under the combination of salinity and heat stress (S+H) treatment resulted in a significant decrease in plant biomass, with reductions of 55%, 60% and 45 % in dry root weights, stems and leaves compared with control plants and when stress was applied individually (Martinez et al., 2018). Second, constraints that have been reported by several authors are the high temperature conditions experienced by crops in the field which are inconsistent with glasshouse or chamber experiments and, even in the field, the temperature conditions vary from year to year and across locations. A study on the effects of temperature was conducted across four production regions in Australia on wheat, barley, oats, chickpeas and field peas which showed that chickpea and field pea yields were affected by high temperatures during grain filling (Dreccer et al., 2018).

The main objective in breeding for heat tolerance is improving the yield trait under stress environments. The first heat tolerance breeding was carried out in cowpeas (*Vigna unguiculata* (L) Walp) (Hall, 1990). The strategy used in this work was the backcross breeding which utilize the cowpea strains TVU4552 and Prima which were found to be heat tolerant. Repeated cross-breeding was carried out between the progenies and their two heat tolerant parents during stages 1 and 2 and CB5 and several other African cowpeas that are heat tolerant during seed coat development and other agronomic traits were incorporated (Hall, 1990) to improve yield component. However, breeding for quality traits to increase their content in food crops is not considered important for legumes as most legumes, including peas, were thought to have sufficient protein that complements the starchy foods such as maize, rice, millet, sorghum, cassava and yam (Singh et al., 2002). But the question would be does selecting for heat stress tolerance reduce protein content? By using this traditional approach, heat tolerant genotypes can be identified amongst the gene pool which can improve quality traits in pea crop under stress environments.

1.8 QUALITY TRAITS

As the world's population is projected to increase to 9.6 billion by 2050 with most of the growth in developing countries, the demand for food production will also need to be raised to sustain the population (Farooq et al., 2018). The rising population will add extra demand on the protein diet as meat products will become more expensive and diet demand for protein will shift. Legumes, like peas and other oil seeds, are a good inclusion to a healthy diet, to provide the protein needed for the ever-increasing population.

A recent study on four field pea genotypes to determine if the varieties differ in their protein content. According to Olle et al., (2015), the crude protein content was lowest around 236 g kg^{1} in dry matter and higher in all other varieties. Similarly, Gbenga-Fabusiwa et al., (2018) conducted an analysis on biscuits produced from pigeon pea flour and wheat flour. Their results showed that the composite biscuits were rich in protein, crude fibre and amylose which suggests that pigeon pea supplement can produce cheap and high-quality products compared to wheat flour. In another study, Mohammed et al. (2018) evaluated six dry pea cultivars across 22 different environments to investigate the pea quality between and amongst environments. They observed a significant difference in protein concentration from 145 to 278 g per kg in seeds, while starch concentration ranged from 439 to 617 g per kg. This study shows the need to develop varieties in terms of high-quality protein and carbohydrates across environments. These findings were similar to Sharma et al. (2015) in a quality trait study, on grain and flour of 14 different field peas, where the authors noted a lower starch content than protein. The Blue value and Kmax were high in protein while accessions with higher amylose showed higher resistant starch (RS), final viscosity, and lower rapidly digestible starch (RDS). Ca, Zn, K and Fe content varied significantly amongst different accessions. The creamish green and white seed accessions showed higher Fe and Zn content. In contrast also based on colour selection and then on seed analysis, that the yellow coloured accessions (1.36–3.71%) showed lower antioxidant activity compared with the brownish and green coloured accessions (4.06– 9.30%). Singh et al. (2002), reviewed several other studies carried out on cowpeas to examine the protein content in several cowpea lines across different countries. Results ranged from 21.5 to 27%, iron from 8 to 15mg/100g dry seeds and newly released cowpea lines, IT83S-728-13 and IT83S-8181 that were used to make snack food in Nigeria called Akara, were found to produce the highest yield and the best Akara due to its high protein value. (Fernandez-Quintela et al., 1997) compared the composition and functional properties of the seeds of three legumes; faba

bean soybean and pea. They found a high protein content in soybeans (36.7%), high carbohydrates were reported in peas (59.4%), and faba bean seeds (52.1%) respectively.

In contrast (Mishra et al., 2010) evaluated thirty genotypes of field peas during rabi cropping season from (2007-2008) for nutrient composition and the analysis revealed a range of proximate principles of the test genotypes. The result shows that protein content ranged from 18.2 to 24.67%, whereas the carbohydrate content was 57.95 to 63.53%. As identified by another study, where five coloured flowering and four coloured flowering polish pea (Pisum sativum) cultivars nutritional usability at full maturity were compared. The study revealed that seeds contain crude protein of up to 224 to 260 g kg^{-1} which was rich in Lys (6.8+0.08g) and tannin presence in white-flowering cultivars was 4.3±0.9 g and in colour-flowering - 7.4±2.2. g (Kotlarz et al., 2011). Moreover, Iqbal et al., (2006) studied the proximity composition, mineral constituents and amino acids profile of four legumes lentils, chickpeas, cowpeas and green peas, and found significant variations existed in all components studied. The lentils contain higher protein (26.1%) followed by green peas (24.9%) while cowpea contained the most ash (4.2%) and crude fat was the highest in chickpeas (5.2%). In a different study to compare the growth of rats, Alonso et al (2002), applied a thermal treatment study on pea seed to improve the nutritional quality. They compared the diets containing raw and extruded peas from the Ballet variety. They observed that growth was reduced by 40% with supplemented raw peas and extruded peas in their diet as the only source of protein. However, when raw pea supplement diet alone was used, faeces, and urinary of nitrogen (N) levels were high in rats fed with raw peas. These various studies provide how important pea crops can contribute to improving protein diets in our daily diets. However, the protein quality can be adversely affected by varying climatic factors such as temperature drought and environmental factors such as salinity which affect grain quality contributing to low quality traits.

1.8.1 Heat effects on grain composition and quality

Most of the legumes like peas are grown in semi-arid, subtropical and temperate regions. Climatic factors like heat, drought and salinity can influence their grain quality. Recently, Farooq et al. (2018) conducted a study on the impact of abiotic stress on nine different legumes and found that heat strongly reduced protein content by 19.6% in peanut at 32/26 °C. In the soybean, protein was greatly reduced when the temperature was increased to 40/30 degrees day/night (Thomas et al., 2003; Wolf et al., 1982). Similarly, reports on soybean grain protein reduction were also noticed when temperatures increased from 14 to 22 °C in studies by Piper and Boote (1999). Koehler et al., (2018) found that higher temperature reduced protein concentrations in soybeans at both the middle and bottom canopy positions while a small effect was found at the top of the canopy. In terms of the oil content, high temperature increased oil contents in all three positions of the canopy in soybean. In peanut and soybean oil content was increased from 37% to 20% (Wolf et al., 1982) under high heat stress conditions. However, Golombek et al. (1995) cited a reduction in oil content in peanuts, while oil quality such as oleic acid content improved under increased temperatures. The temperature effect on kidney beans reduced the oil content by 23% (Thomas et al., 2009) with similar results reported in soybean at 35 °C and 29 °C respectively (Dornbos & Mullen, 1992). Furthermore, in soybean, when the temperature was increased from 28/18 °C to 44/34 °C, it negatively affected the nitrogen (N), phosphorus (P), starch total oil, fatty acids and carbohydrate content (Humphreys, Canter, & Thomas, 2003). The proximate analysis of the seed reserves in lentil cultivars also showed that heat stress reduced protein by 26-41%, starch by 25-43% and fat by 39-57% while it increased total sugars by 36-68% relative to control (Sita et al., 2018). Accumulation of many of the storage proteins such as albumins, globulins, prolamins and glutelins (22–42%) were also inhibited and amino acids decreased significantly under heat stress in comparison to the control. However, some proteins such as proline, glycine, alanine, isoleucine, leucine and lysine, increased. Heat stress reduced mineral content such as Fe (17–52%), Ca (13–28%), P(10–54%), K (12.4–28.3%) and Zn (36–59%) in seeds, compared with controls (Dürr et al., 2018).

In addition, Zhou et al. (2018) compared the total fatty acid and fatty acid compositions of two oil rape seed cultivars under low and high temperatures. The result shows that the total fatty acid and fatty acid compositions under the two temperature regimes were significantly reduced by 18.9% to 13.7% in total fatty acids. In a similar study, Dürr et al. (2018) conducted a controlled environment experiment to assess the effects of high temperature between 25 and 35°C during grain filling in peas and wheat and reported that protein and total fatty acid concentration increased when stress occurred during grain filling. A high content of vicilins was recorded in peas while increase polyunsaturated fatty acids were noted in both crops. The total starch was decreased in wheat along with sucrose in both crops.

The higher temperature also had a profound effect on non-structural carbohydrates by decreasing the concentration and this was evident in soybean grains (Farooq et al., 2018). Higher temperatures decreased fructose and glucose while increasing sucrose and rafinose. However, the peanut sucrose content declined by 56% when the temperature was 32/26 °C (Humphreys et al., 2003). High temperatures have negative effects on oil content, protein, fatty acids and sugar content in most legumes including pea.

CHAPTER 2

2.1 MATERIALS & METHODS

The present study was conducted at the Plant and Growth Unit (PGU - (40°22 55" S, 175°36'22" E) at Massey University, Palmerston North. Six pea cultivars Sugar snap dwarf, Organic pea progress, pea Alderman tall climbing, Greenfeast, Snow pea, and WF Massey were initially grown. The first three were sourced from Kings Seeds (NZ) Limited and last three were from Terranova Seed PTY Limited, Auckland, New Zealand. The first initial germination test (4 x 50 seeds germinated at 20°C for 8 days in paper rolls) was initiated in May 2018 at the School of Agriculture and Environment (SAE) seed laboratory. During the germination test, one of the cultivars (Alderman) were discovered to contain a dead pea weevil (*Bruchus pisorum*) which is a prohibited species in New Zealand. As a precaution the seed lot was destroyed by the relevant authorities. The remaining five cultivars were sown into the two separate controlled glasshouses on the 15th of May 2018 in 8 litre pots. The pots were filled with standard soil mixture which consisted of 200grams of long-term mix fertilizer, 100 grams of short-term mix fertilizer[2] and 150 grams of dolomite lime. Thirty-nine (39) days after planting (DAP) we realised that cultivars WF Massey, Pea organic progress and Sugar snap dwarf were starting to flower and pods started forming. Therefore, data was only collected from the two late flowering cultivars (Greenfeast and Snow pea). These two seed lots were chosen because they can mature between 65-90 days. Maturity is the time when all pea seeds are ready to harvest for eating while for seeds peas are left to dry and then harvested.

Table 1 Cultivars used with their origin, height and days to maturity days

Cultivar types	Origin	Days to germination	Height (cm)	Days to maturity
Greenfeast	Australia	7- 10	150	75-85
Snow pea	USA	7-14	60	55-65

[2] Both Short and Long term fertilizers were Osmocote brand, 3 & 6 month brands respectively . See http://osmocote.co.za/

2.1.1 Glasshouse experiment

The experiment was performed in two temperature regulated glasshouse environments labelled 18 and 16 with two pea (*Pisum Sativum*) cultivars (Greenfeast) and (Snow pea). As highlighted in the previous section where some cultivars were discarded early before the stress was applied. The glasshouses are equal in size: 8m x 6m. The experiment design was a factorial design layout in a randomised complete block design (RCBD) with four replicates. Three seeds of each cultivar were sown in each 8 L pots in each glasshouse with each cultivar having 3 data plants per pot in each replicate. The treatments were cultivars (2) and temperature: one low temperature glasshouse (25/15°C) and one high temperature glasshouse (35/25°C). The third treatment was based on the pod set dates (2). Pod set date one was 30 July-10 August 2018 and pod set date two was 24 August to 31 August 2018. The pod set dates are only for the glasshouse 16 where the crops were exposed to the high temperature range (35/25°C). Pod sets after 27 August 2018 were not included. The pod set dates are used to compare the effect of temperature on the seed quality.

At the start of the experiment both glasshouses 18 and 16 were at the same temperature 15/25°C day/night. The plants used as non–stress or control continued to grow in glasshouse 18 at 25/15°C day/night. Glasshouse 16 was the high (hot) temperature glasshouse (35/25 °C/day/night). After 2 weeks (DAP) the glasshouse temperature was increased from 25/15 °C to 26/18 °C and was maintained at this temperature until stress was applied. On 24 August 2018 glasshouse 16 was exposed to higher temperature heat stress at (35/25°C) day /night temperature when almost 50 % of the plants in the glasshouse were observed to be flowering and (anthesis) pod development as this is the critical stage for pod-filling (Figure 2). In addition, normal agronomic management practices were applied such as weeding and watering of the plants.

2.1.2 Climatic conditions

The mean temperature and relative humidity (RH) in the glasshouse were recorded throughout the experiment using a data logger placed inside and above the plants in the glasshouse. Rainfall data were collected from the Grassland Research Weather Station. The temperature and RH were recorded every 10 minutes. Drip irrigation was applied every two hours in the glasshouse 16 daily to maintain field capacity and avoid interaction between heat and drought stress while glasshouse 18 water was rescheduled every four hours. The high temperature (35/25 °C) was

applied on 24 August 2018 in the (HT) glasshouse (16). After 24 August 2018 the maximum temperature attained was between 30 °C and 35 °C, however after 17 September 2018 minimum glass house temperatures could not be maintained above 20 °C.

Figure 1: Pea plants growing under the two temperature regimes with glasshouse 16 (top) and glasshouse 18 (bottom).

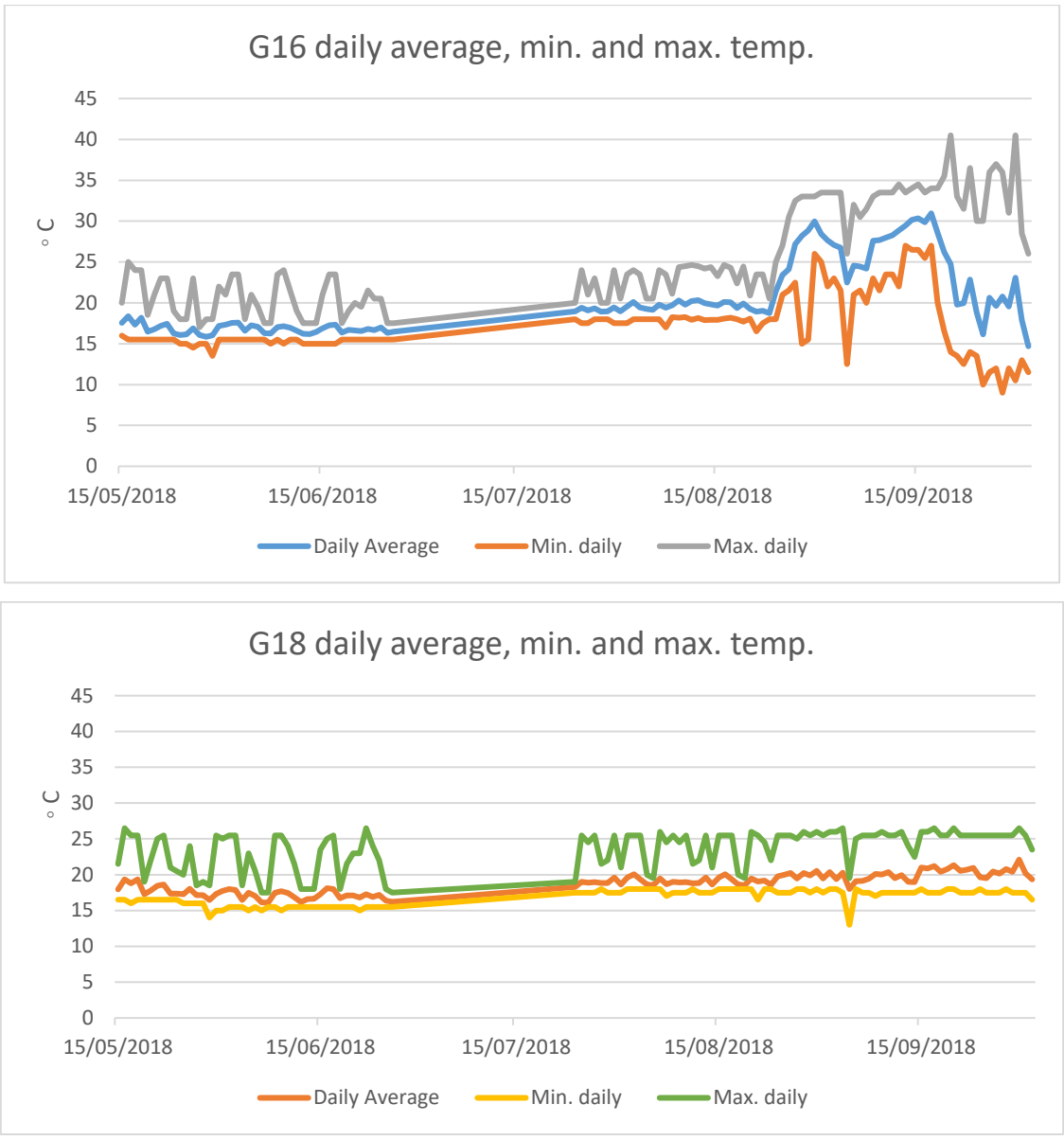

Figure 2 Average, minimum and maximum temperatures in glasshouse 16 (G16) and glasshouse 18 (G18) for the duration of the experiments.

2.1.3 Yield Components

2.1.3.1 Number of pods per plant

Total number of pods per plant was determined every 3 days by counting the number of pods developed on each cultivar and grouping them according to dates of flowering. A letter was assigned from A-P for different pods developed at each different date. The pods set number at harvest was also counted. This is denoted as (PSN**). PSN** only refers to the number of pods harvested at final harvest.

2.1.3.2 Flower number

Total flower number was counted during tagging of the flowers from each cultivar according to date groupings.

2.1.3.3 Number of seeds per pod

The number of seeds was determined by shelling the pods harvested and counting all the seeds. We took this approach has there were not enough pods to and collect the required number of seeds needed than the previous studies where they take a representative sample of the pods at random to count the number of pods (Shukla, 2015)

2.1.3.4 Plant height

Plant height was measured from the ground level to the tip and the average plant height was calculated.

2.1.3.5 Seeds weight (g)

Seeds were removed from the pods and counted, and weight was recorded in grams. All seeds whether deformed or small in size. All were counted and their weights recorded.

2.2 GERMINATION

Prior to the standard germination test, seeds were air dried at ambient room temperature in the PGU laboratory lecture room, which is well ventilated with no direct sunlight, to 14-15% moisture using a digital moisture meter as described by Shinohara et al. (2006a) and Hill (1999). The seeds were stored in the ambient temperature until they reached the required moisture content (14-15%). They were then stored in paper bags and retained in the ambient environment condition until they were used for quality testing.

The germination test was performed on both cultivars from both temperature regimes using the standard germination test (ISTA, 2018) immediately after harvest. Four replicates of 50 seeds were germinated between pea paper (38 lb. Regular Weight Seed Germination Paper, Anchor Paper Company Ltd, Minnesota) for 5 days and 8 days respectively at 20 °C. Seedlings were evaluated and classified into normal, abnormal, hard seeds, death seeds using the description of Castillo et al. (1993a), and ISTA (2018) . The data obtain were expressed as percentages

$$\text{P} \quad (\%) = \frac{\text{Number of germinated seeds in final count}}{\text{Number of total seed (50)seeds}} * 100$$

2.2.1 Hollow heart

The hollow heart was determined using the normal seedling counts from the germination test. However, because of the limited number of seeds from some samplings from the glasshouse 16 less than 20 seeds per seed lot were used for the test. The seeds of the normal seedlings were dissected into half by breaking the cotyledons into half by hand to expose the adaxial surfaces (Hampton & Scott,1982, A. G. Castillo et al., 1993; Hampton & Scott, 1982; Shinohara et al., 2006a). The presence of depressions in either or both cotyledons was recorded as hollow heart. The data obtain were expressed as percentages based on the total number of seeds evaluated in the germination test.

2.3 QUALITY TRAITS

2.3.1 Protein and Total Sugars

The protein content in the two pea cultivars were analysed at the Massey Institute of Food Science and Technology Nutrition Laboratory using the AOAC 96.06 (Dumas method) where the N-P factor is 6.25. In total 32 samples were submitted for the analysis. However, due to low number of seeds from some of the treatments the target of 10 peas per cultivars was not used for each treatment.

2.3.2 Protein Analysis

The protein analysis was performed using Dumas method as described by Shotts et al. (2018). The samples were weight to 200milligrams in tin capsules and were introduced into the combustion reactor where the combustion digestion of the samples takes place at 950 °C in excess oxygen. After combustion the produce gasses are collected and pass through several straps and are removed except nitrogen and nitrogen oxides. The nitrogen is transferred into a molecular nitrogen and nitric acid (10). After going through several stages. The nitrogen oxide was converted into elemental nitrogen and collects the oxygen in access while different traps remove the residual water and carbon dioxide After a two-stage drying phase, the total nitrogen content is measured by thermo conductivity detector via an electronic flow controller. A

connected PC calculates the nitrogen concentration in the sample from the TCD signal of the N2 in the CO2 and from the sample weight. The content of crude protein is calculated by multiplying the measured nitrogen quantity by the appropriate factor (6.25) and is expressed in percent (OAC Official Method, 2012, 992.15; Shotts et al., 2018).

Firstly, the samples were freeze dried at low temperature. The samples were then grounded into powder form and 200 milligrams per treatment were submitted for the analysis. The analysis was performed using a Rapid N exceed analyser. The samples were weighed on a balance to around 1gram which is connected to the computer. The sample was then placed through the combustion chamber with a standard blank sample (see Fig.3). The Nitrogen reading is than uploaded quickly into the computer. A protein factor of 6.25 was then applied by multiplying the Nitrogen value against the protein factor to determine the actual protein content in pea (%) (GmbH, 2016).

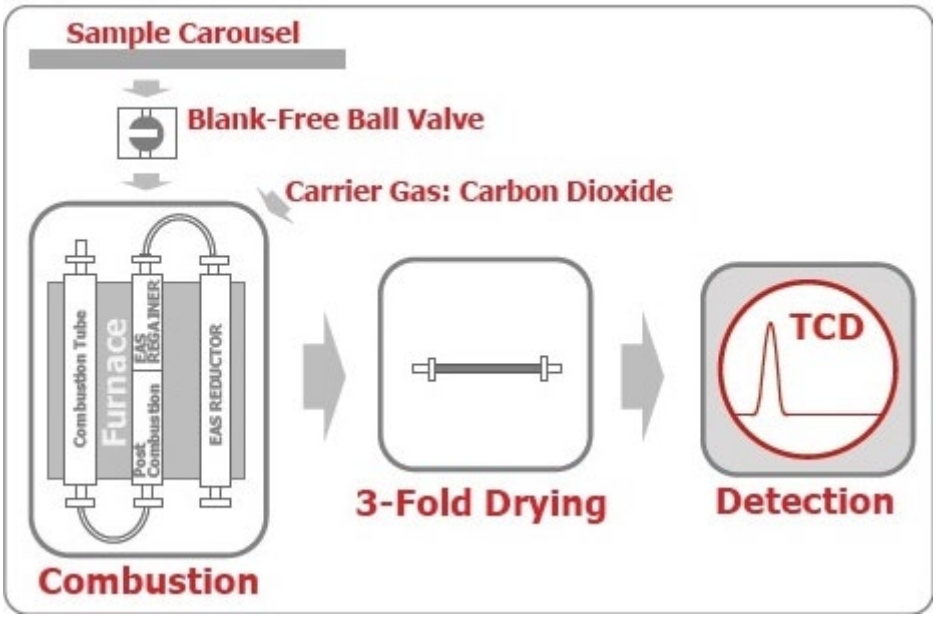

Figure 2 Functional principle of the rapid N exceed by Dumas Method (source: Elementar Analysensysteme Gmb, 2016).

2.3.3 Total Sugars

The total sugar was determined using the phenol sulphuric method described by (Vadhaman et al., 2017). This method is chosen because it is more reliable amongst other quantitative assays. The main reagents used in this method are 96% sulphuric acid, standard glucose, 2,5 NHCL, sodium carbonate, distilled water and phenol solution. After grinding of the samples into

powdered form, about 100mg of the glucose were placed into each test tube. Then 5ml of 2.5 NHC1 was added and boiled in water bath for 3 hours to hydrolyse the sugar. These sugars were then left too cool under room temperature. After this sodium carbonate was added and about 100ml was pipetted out to make working standard which were placed into series of test tube according each replicate while the blank was set with all the reagents but no sample. Following this, about 1 ml of the phenol solution was added into each tube than followed by adding 5ml of sulphuric acid 96% and shake thoroughly and were placed aside for 10 minutes. Each test tube was placed into the water bath at 25-30C for 20 minutes. The spectrometer was switch on and the absorbance (optical density (OD)) of blank was adjusted to make it zero.

2.3.4 Statistical Analysis

A nonlinear regression model (NLIN Procedure) fitting data with a unimodal distribution was applied to model the number of flowers or pods (y) over the growing season: $y = y_{asym}/\{1+[(t-t_m)/c]^2\}$, where y_{asym} is the maximum y at time (days, t_m), c is a constant rate of the curve (Figures 1 and 2). A Verhulst's (1838) logistic asymptotic regression model (NLIN Procedure) was applied to fit the data of cumulative proportion of flower or pop number (y) over the growing season (Figures 1 and 2): $y = y_0+y_{asym}/\{1+\exp[-k(t-t_m)]\}$, where, y_0 and y_{asym} are the initial and asymptotic y value, respectively; t is the time (e.g., days) since seed sown, t_m is the inflection time point at which the growth rate is maximized, k determines the steepness of the curve (see Archontoulis & Miguez 2015). Those estimated parameters were significantly different from 0 if the 95% confidence limits (CL) did not include 0 (i.e., both up and low CLs > 0 or < 0). The coefficients of determination (R^2) for regressions were calculated as: 1 – (residual sum of square/corrected total sum of square). The difference in each estimated parameter was compared between the combination treatments of cultivar and temperature according to Juliano (2004): if the 95% CLs overlap, there is no significant difference.

The normality of other data presented in Figures 3-6 was tested using a Shapiro-Wilk test (UNIVARIATE Procedure) before analysis. Data on the total number of flowers and pods (Figure 3), sugar content (Figure 4b), seed germination at day 5 (Figure 5a) and shoot dry weight (Figure 6a) were normally distributed and thus analyzed by an analysis of variance (ANOVA, GLM Procedure) followed by a Tukey's studentized range test. Data on protein content (Figure 4a), seed germination at day 8 (Figure 5b) and root dry weight (Figure 6b) were not normally distributed even after transformation, and hence analyzed using nonparametric ANOVA followed by Tukey's studentized range test for multiple comparisons.

CHAPTER 3

3.1 RESULTS

We initiated the first initial germination test in May 2018 at the School of Agriculture and Environment (SAE) seed laboratory. After the test, one of the cultivars (Alderman) was discovered to contain a dead pea weevil (*Bruchus pisorum*) which is a prohibited species in New Zealand. As a precaution the seed lot was destroyed by the relevant authorities. As part of the security measure by Ministry for Primary Industries (MPI), the pea weevil infected seed was packaged and air freighted to MPI in Wellington for further diagnosis and confirmation. The results show that the insect was dead before the fumigation and before it reached New Zealand (MPI, personnel communication, May 2018).

The normality of other data presented in Figures 3-6 was tested using a Shapiro-Wilk test (UNIVARIATE Procedure) before analysis. Data on the total number of flowers and pods (Figure 3), sugar content (Figure 4b), seed germination at day 5 (Figure 5a) and shoot dry weight (Figure 6a) were normally distributed and thus analyzed by an analysis of variance (ANOVA, GLM Procedure) followed by a Tukey's studentized range test. Data on protein content (Figure 4a), seed germination at day 8 (Figure 5b) and root dry weight (Figure 6b) were not normally distributed even after transformation, and hence analyzed using nonparametric ANOVA followed by Tukey's studentized range test for multiple comparisons.

3.1.1 Flower and pod number accumulation

As shown in Figure 4 and Table 2, flower number of Greenfeast peaked at day 102.3 at 25/15 ºC which was significantly later than that of Greenfeast at 25/15-35/25 ºC (day 89.2) and Snow pea under both temperature regimes (day 89.4 and 88.0), and the rate of increase was significantly greater for Greenfeast at 25/15 ºC than Snow pea.

Greenfeast at 25/15ºC had a significantly lower flower accumulation rate (k) than other treatments; the maximum rate of flower accumulation for Greenfeast at 25/15 ºC was detected at day 96.6 with which was significantly later than other treatments (Figure 4 and Table 3).
Snow pea at 25/15 °C had a significantly higher flower accumulation rate (k) than Snow pea at 25/15-25/35 °C.

Figure 4 and Table 2, pod number of Greenfeast was significantly higher at day 104.10 at 15/15 °C than Greenfeast at 15/25-35/25 °C while Snow pea pod number was significantly higher at day 91.97 at 25/15 °C than at 25/15-35/25 °C and the rate of increase was higher for the Greenfeast at 25/15 °C, followed by Snow pea t 25/15-35/25 °C with the highest correlation (R^2=0.73).

Furthermore, Greenfeast at 25/15 °C pod had a significantly lower pod accumulation rate (*k*) than other treatments; likewise, the maximum rate for Greenfeast pod accumulation at 25/15 °C was detected at day 98.73 with which was significantly later than other treatments (Figure 2 and Table 2). The highest correlations are detected between pod accumulation rate (*k*) Snow pea at 25/15 °C and Greenfeast at 25/15-35/25 °C (R2=0.96) respectively (Figure 4 and Table 3).

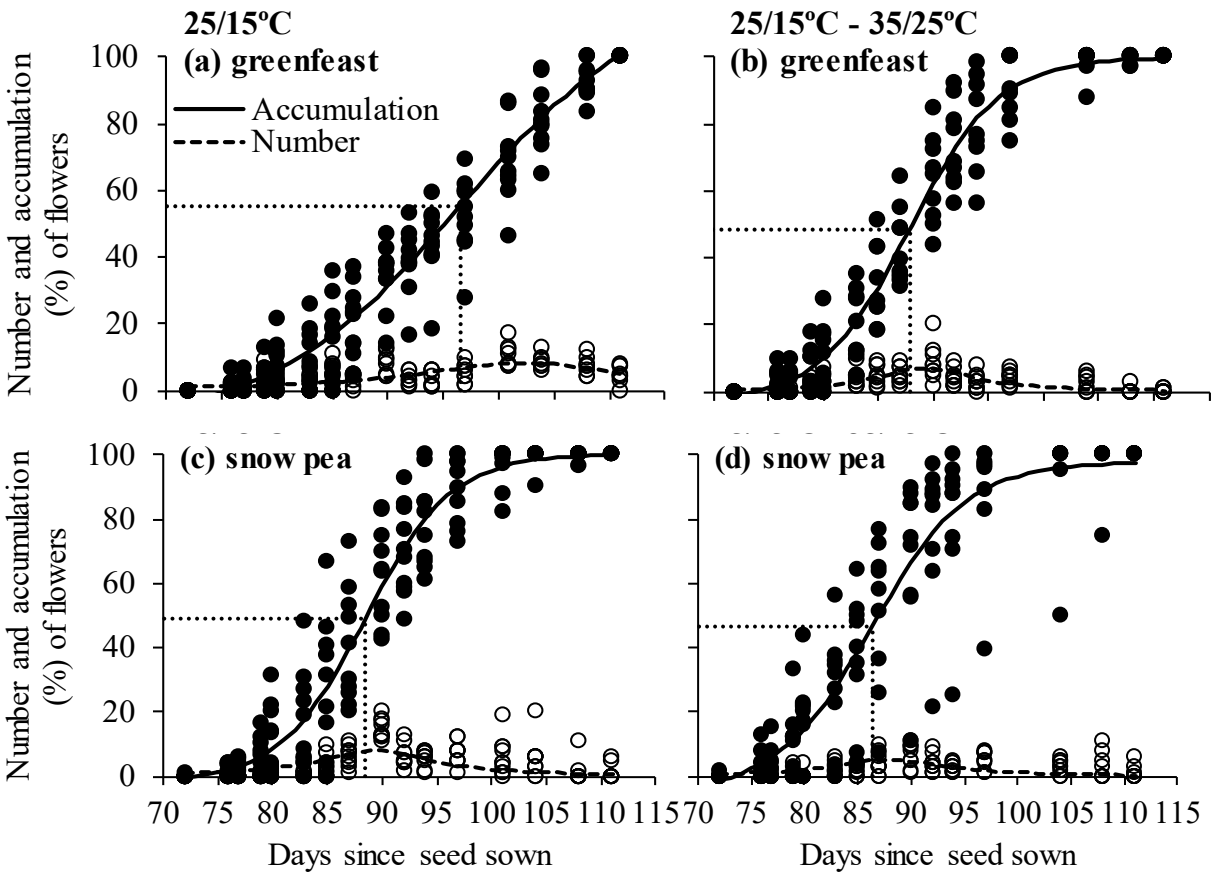

Figure 3 Population dynamics of Greenfeast and Snow pea flowers at different environmental conditions. The dotted lines indicate the inflection time point at which the growth rate of accumulation is maximized.

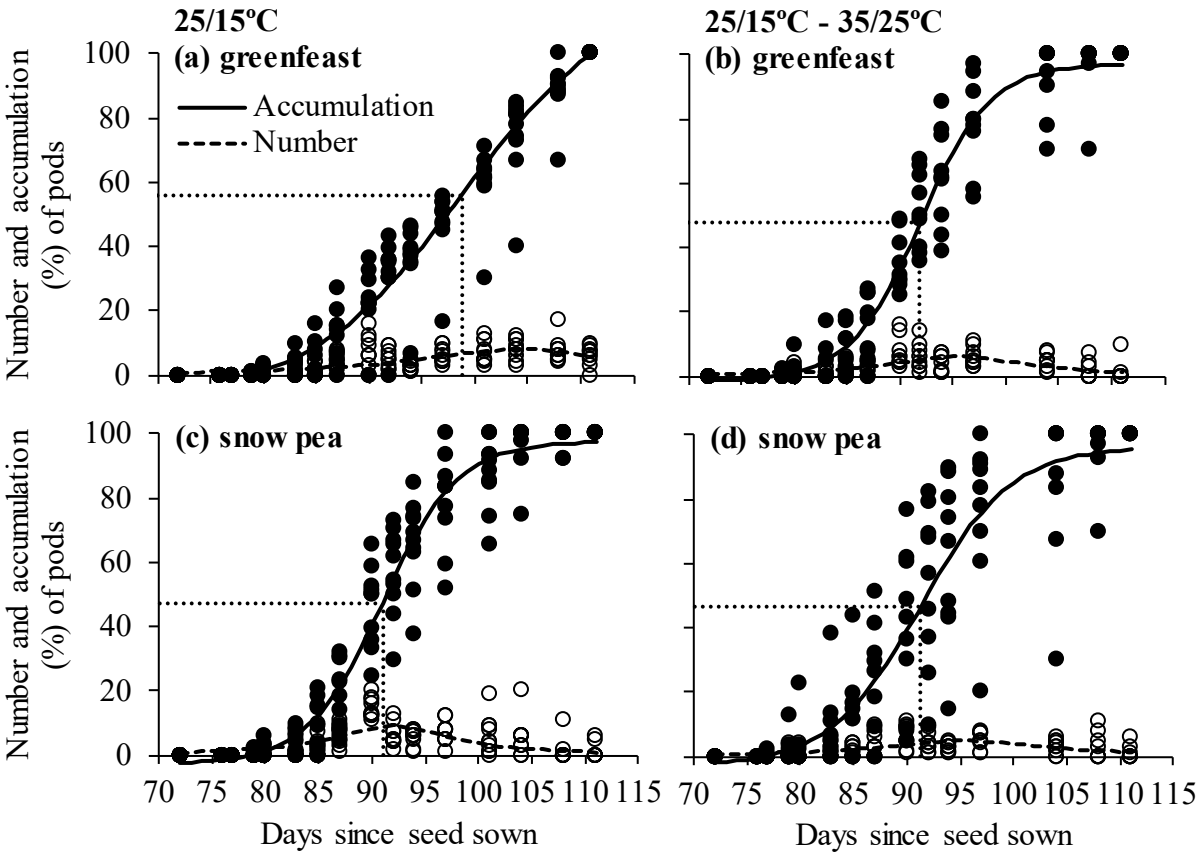

Figure 4 Population dynamics of Greenfeast and Snow pea pods at different environmental conditions. The dotted lines indicate the inflection time point at which the growth rate of accumulation is maximized.

Table 2 Summary of results of modelling number of Greenfeast and Snow pea flowers and pods.

Temp (°C)	Cultivar	y_{asym}	t_m	c	R^2	$F_{(df)}$*
Flower						
25/15	Greenfeast	8.42±0.58	102.30±0.89 a	11.61±1.38 a	0.7359	$145.83_{(3,157)}$
	Snow pea	8.01±0.72	89.41±0.56 b	6.58±0.90 b	0.6302	$89.20_{(3,157)}$
25/15-35/25	Greenfeast	7.05±0.64	89.19±0.47 b	5.66±0.78 b	0.6764	$91.99_{(3,132)}$
	Snow pea	5.21±0.49	87.97±0.59 b	6.74±0.98 b	0.6544	$83.29_{(3,132)}$
Pod						
25/15	Greenfeast	8.15±0.56	104.10±0.96 a	11.53±1.42 a	0.7293	$141.01_{(3,157)}$
	Snow pea	9.22±0.63	91.97±0.63 c	6.70±0.87 b	0.6224	$86.25_{(3,157)}$
25/15-35/25	Greenfeast	6.20±0.62	95.45±0.95 b	8.16±1.18 ab	0.6025	$66.68_{(3,132)}$
	Snow pea	4.89±0.52	95.28±1.12 b	10.14±1.60 a	0.5761	$59.79_{(3,132)}$

* $P < 0.0001$ for all models. Model: $y = y_{asym}/\{1+[(t-t_m)/c]^2\}$. For flower or pod, mean t_m or c with the same letters are not significantly different (95% CLs overlap).

Table 3 Summary of results of modelling accumulation of Greenfeast and Snow pea flowers and pods.

Temp (ºC)	Cultivar	y_0	y_{asym}	t_m	k	R^2	$F_{(df)}$*
Flower							
25/15	Greenfeast	-10.67±13.94	132.54±42.13	96.57±3.30 a	0.12±0.05 b	0.6768	$108.90_{(1,156)}$
	Snow pea	-2.00±2.69	102.35±3.82	88.43±0.40 b	0.26±0.02 a	0.9312	$703.89_{(1,156)}$
25/15-35/25	Greenfeast	-2.98±2.48	103.09±3.45	87.81±0.36 b	0.25±0.02 a	0.9545	$915.55_{(1,131)}$
	Snow pea	-5.12±6.06	103.01±7.83	86.29±0.81 b	0.23±0.04 a	0.8482	$244.03_{(1,131)}$
Pod							
25/15	Greenfeast	-8.00±9.78	127.84±39.36	98.73±3.40 a	0.14±0.05 b	0.6329	$89.65_{(1,156)}$
	Snow pea	-2.64±1.54	100.28±2.53	91.01±0.27 b	0.29±0.02 a	0.9578	$1179.36_{(1,156)}$
25/15-35/25	Greenfeast	-1.37±1.45	98.66±2.50	91.89±0.27 b	0.30±0.02 a	0.9568	$966.12_{(1,131)}$
	Snow pea	-2.93±3.61	99.42±6.02	91.29±0.65 b	0.23±0.04 a	0.8527	$252.85_{(1,131)}$

* $P < 0.0001$ for all models. Model: $y = y_0 + y_{asym}/\{1+\exp[-k(t-t_m)]\}$. For flower or pod, mean t_m or k with the same letters are not significantly different (95% CLs overlap).

For each cultivar, the total number of flower and pods were significantly higher (excepting Snow pea at 25/15 ºC compared with Greenfeast at 25/15-35/25 ºC) at 25/15 ºC than at 25/15-35/25 ºC ($F_{3,34} = 4.72$ and 5.69 for flower and pod, respectively; $P < 0.01$) with no significant difference detected between cultivars for each temperature type (Table 3).

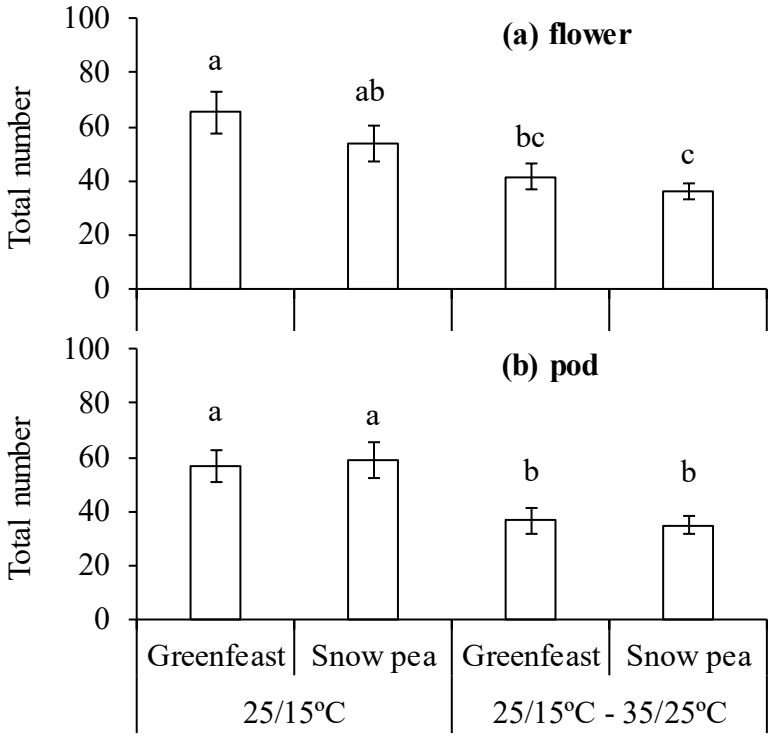

Figure 5 Total number of flowers and pods of Greenfeast and Snow pea at different environmental conditions. Columns with the same letters are not significantly different ($P > 0.05$).

3.2 SEED GERMINATION

There were significantly more Snow pea seeds germinated at 25/15 ºC in the first 5 days ($F_{3,28}$ = 49.74, P < 0.0001) (Figure 7a), while the final germination rate was not significant between combination treatments ($F_{3,28}$ = 0.92, P = 0.4421) (Figure 7b).

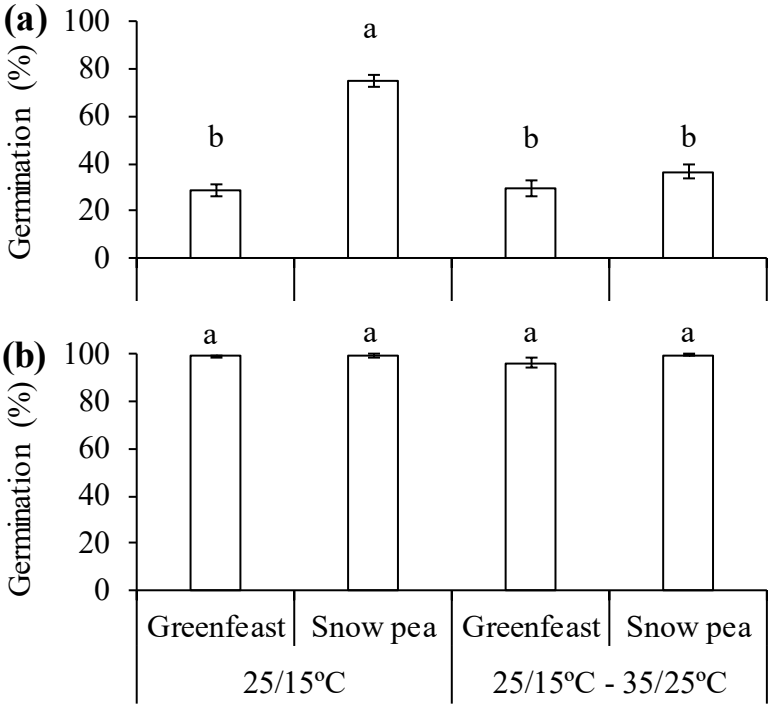

Figure 6 Proportion of Greenfeast and Snow pea seeds harvested from different environmental conditions germinated at day 5 (a) and day 8 (b). Columns with the same letters are not significantly different (P > 0.05).

3.2.1 Shoot and root dry weights

Snow pea had significantly higher shoot dry weight at 25/15 ºC than Snow pea at 25/15 ºC - 35/25 ºC and Greenfeast at 25/15 ºC with significant lower shoot dry weight detected for Greenfeast at 25/15 ºC ($F_{3,28} = 24.86$, $P < 0.0001$) (Figure 8a). Root dry weight of both cultivars was significantly higher at 25/15 ºC than that of Greenfeast at 25/15-35/25 ºC ($F_{3,28} = 6.08$, $P = 0.0026$) (Figure 8b).

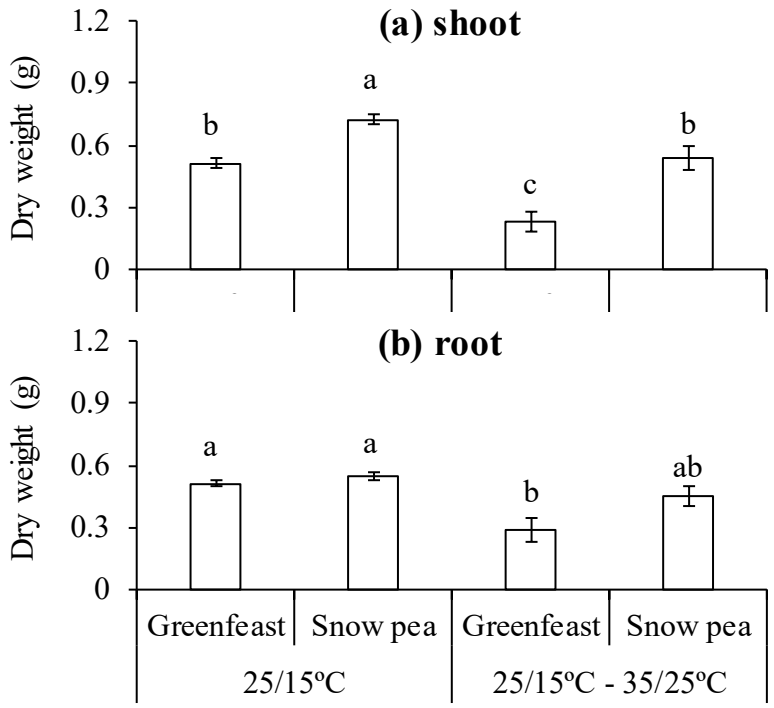

Figure 7 Dry weight of shoot (a) and root (b) of Greenfeast and Snow pea at different environmental conditions. Columns with the same letters are not significantly different ($P > 0.05$).

3.3 QUALITY ANALYSIS

3.3.1 Protein and Sugar levels

Greenfeast had significantly higher protein content than Snow pea at 25/15 ºC ($F_{3,28} = 4.28$, $P = 0.0132$) (Figure 9a) and significantly higher sugar content than Snow pea at each temperature type ($F_{3,28} = 13.38$, $P < 0.0001$) (Figure 9b).

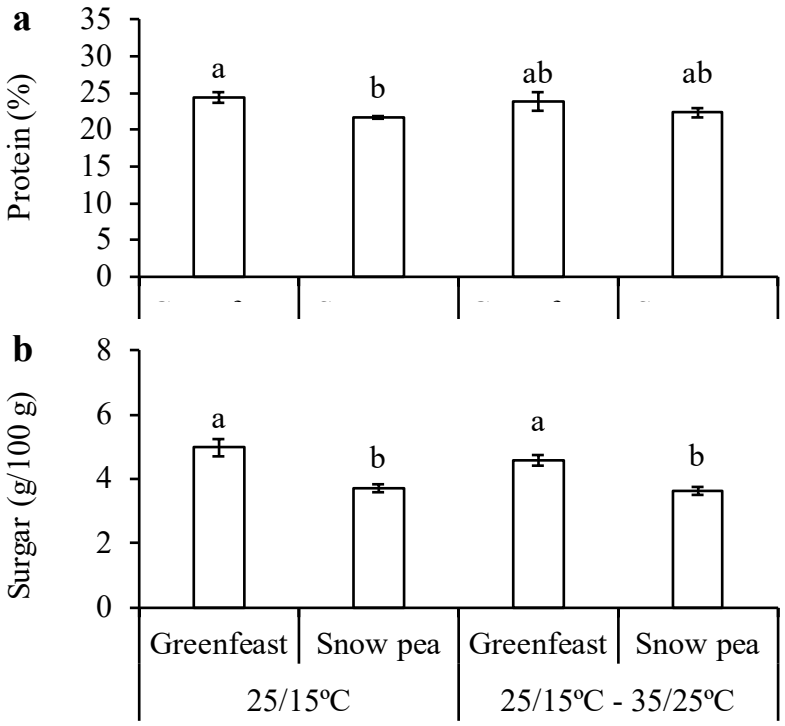

Figure 9 Protein and sugar content in seeds of Greenfeast and Snow pea at different environmental conditions. Columns with the same letters are not significantly different ($P > 0.05$).

CHAPTER 4

4.0 GENERAL DISCUSSION

Pea is an important economic pulse crop in New Zealand (Millner & Roskruge, 2013). Research and breeding programmes into pea have been widely documented in New Zealand since the early introduction of pea into the country (Jermyn, 1987; Martin et al., 2008). The pea breeding objective in New Zealand is based on breeding for resistant to disease, high seed quality and yield (Jermyn, 1987). The breeding for tolerance to heat stress or temperature stress was not a primary objective of the breeding programmes. However, during warmer months in spring and summer pea yield and quality were affected. Therefore, this study was conducted to determine the effect of increased temperature on pea production and pea seed quality using cultivars sold commercially in New Zealand.

The pea seeds were sourced from two New Zealand based commercial seed companies, Terranova Seeds Ltd and Kings Seed. Ltd The experimental design used was completely randomised design (RCBD). The seeds after harvest were subjected to seed germination. Seed vigour was assessed using interim germination counts and by measuring root and shoot dry weights after 8 days seedling growth).

4.1 FLOWER AND POD ACCUMULATION NUMBER

The maximum production of pea depends on the number of flowers produced and pods developed. It was documented in several legumes species that high temperature is lethal to flower buds both female and male reproductive organs of many legumes (Kaushal et al., 2016), common beans (Gross & Kigel, 1994), chickpeas (Kaushal et al., 2013; Kumar et al., 2013), and peas (Guilioni, 1997). This study was able to demonstrate that the temperature plays an important role in reproductive organs starting from flower initiation through to seed development respond differently to high temperature stress (Sita et al., 2017). The flower number showed that plants subjected to high temperature (25/15-35/25 °C) significantly reduced the number of flowers compared with the 25/15 °C temperature regime. This shows that most of the flowers were aborted before being fully developed. High temperature during flowering period was observed to reduce pod formation and similar observation were reported by Devasirvatham et al. (2013) in chickpeas. The flower size and deformation of floral organs was observed in the high temperature (25/15-35/25 °C) regime consequently contributing to

loss of flower and young pods. Our observation was similar to those found in chickpea and mung beans (Sita et al., 2017). In addition, the pod numbers under high temperature 25/15-35/25 °C reduced pod numbers and pod accumulation rate (k) at day 91.3 to 91.9 respectively compared with 25/15 °C. In terms of the pod flower accumulation number, the highest number of flower accumulation was detected in Greenfeast at day 96.6 whilst a lower accumulation number was recorded under high temperature 25/15-35/25 °C regime. Our findings are supported by Kaushal et al, 2016, Gupta et al, 2015, and Sita et al., 2017, in chickpea and common bean. High temperature reduces flower number, flower accumulation number, pod number and pod accumulation number.

4.1.2 Seed germination

To produce high quality seed lots, germination is an important criterion which can be defined as process that begins with imbibition and which is completed by the production of normal seedlings. Germination is desired by the seed industry to be close to 100 % (ISTA, 2018). Seed germination can be affected by various factors including harvesting, threshing, storage, cleaning and drying (Padrit, 1996). However, both Egli et al. (2005a) and Hampton et al. (2013) reported that the seed lot can also be affect by temperature, rainfall and relative humidity. High temperature during seed filling disrupts seed development which increased the proportion of shrivelled seeds resulting in low seed germination.

In this present investigation, we investigated the effects of high temperature on seed germination in two different pea cultivars as the optimum temperature that can encourage seed germination and development is 13-20 °C and 15-25 °C respectively which are also accepted as the threshold for pea germination and growth in agreement with Sita et al. (2017). The result of the study has shown that germination rate at 5 days was in favour of Snow pea compared with Greenfeast at 25/15 °C whereas for final germination percentage the cultivars show no significant difference (Figures 4a and b). Greenfeast had the lowest interim germination at 25/15 °C and 25/15-35/25 °C with only 29% germinating. Though the Snow pea had the highest interim germination. This study is in consistent with study conducted on two pea cultivars; that shows no significant difference under high temperature treatments (Nandagopalan, 2017; Sita et al., 2017). It was noticed that shoot dry weight was highest at 25/15 °C in Snow pea and then Snow pea at 25/15-35/25 °C combined treatments. Greenfeast show the lowest shoot dry weight at 25/15 ºC compared with the root dry weight which is higher for both cultivars at 25/25 °C than that of Greenfeast at 25/15-35/25 °C. Overall, shoot and root dry weight was also reduced

under high temperature (25/15-35/25 °C). A similar finding in seed dry weights under increased temperature was reported by Prasad and Djanaguiraman (2014) to 30/40 °C on floret fertility and grain weight in wheat. Seed germination shoot dry weight and root dry weights were reduced in both cultivars under high temperature.

4.1.3 Protein and Total Sugars

The total protein content was highest for Greenfeast at 25/15 °C and also in combination treatments 25/15-35/25 °C with the total protein range from 24 % to 23 % respectively. Likewise, for the Snow pea, the protein content ranges from 21.69 at 25/15 °C to 22.3 at 25/15-35/25 °C treatments. The protein contents are similar to previous study by (Padrit, 1996). He reported that the total protein content in pea genotypes range from 18 to 24.7 %. This is also similar to study review by (Padrit, 1996) found 224 to 260 kg crude protein on legumes. The similar finding were reported by (Mishra, Dubey, & Rao, 2010; Rodrigues et al.,2012) on peas which range from 22.8 to 26.1 %.

As shown in figure 9b, Greenfeast had the highest sugar content at 25/15 and 25/15-35/25 °C but were not significantly different. The sugar content ranges from 4.9 to 4.5 g/100 g in Greenfeast and 3.7 and 3.6 g/100g in Snow pea. Our findings were similar to (Rodrigues et al., 2012) he reported that total sugar range from 7.9 % to 9.4 % to 3.7% to 5 %. The result of this study had shown that protein and sugar content can be affected by high temperature. This is in agreement with study by (Harsh et al., 2016) who reported that mild or moderate stress can reduce sugar content. The effect of higher temperature on protein was investigated in soybean from middle, top and bottom and the result shows that protein was reduced in seed from the middle and bottom canopy positions while a minute effect was detected at the top canopy (Boote et al., (2018). Farooq et al. (2018) reported that high temperature strongly reduced protein content by 19.6 % in peanut at 32/26 °C and similarly, protein was greatly reduced when the temperature was increased to 40/30 degrees day/night (Thomas et al., 2003; Wolf et al., 1982), soybean grain protein reduction were also noticed when temperatures increased from 14 to 22 °C in studies by Piper and Boote (1999). Boote et al., (2018) found that higher temperature reduced protein concentrations in soybeans at both the middle and bottom canopy positions while a small effect was found at the top of the canopy. In addition, when the temperature was increased from 28/18 °C to 44/34 °C, it negatively affected the nitrogen (N), phosphorus (P), starch total oil, fatty acids and carbohydrate content in soybean (Humphreys, Canter, & Thomas, 2003). The proximate analysis of the seed reserves in lentil cultivars also

showed that heat stress reduced protein by 26-41 %, starch by 25-43 % and fat by 39-57 % while it increased total sugars by 36-68 % relative to control (Sita et al., 2018).

CHAPTER 5

5.1 CONCLUSIONS

The application of the nonlinear regression model (NLIN Procedure), the Univariate procedure and the GLM procedures combined in analysing the data provided a useful approach to an unbalanced data.

High temperature drastically reduces male and female reproductive organs by reducing flower number and pod number. The flower number, flower accumulation rate (k), pod number, pod accumulation rate (k) were reduced under the high temperature growth environment. The interim seed germination, shoot dry weight and root dry weight were highest under the 25/15 °C compared to 25/15-35/25 °C. The total sugar content was in favour of Greenfeast irrespective of the two different temperature regimes.

5.2 RECOMMENDATIONS

- This study should be repeated in the future but must look at pollen viability and pollen tube germination in peas under high temperature under control and field condition
- Breeding should be conducted in the glasshouse under high temperature condition before the seeds can be selected and evaluated under both field and glasshouse condition.
- It is recommended that field trials should be done around September to November in the summer where temperatures are above 25 °C.
- It should be recommended that future study should consider drought and high temperature effects together on flowering and pod development of peas under glasshouse and field conditions

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PERSONAL COMMUNICATIONS

A. Russell, personnel communication, September 1st, 2017

MPI, personnel communication, May 2018

APPENDICES

APPENDIX 1.

MEAN SUMS OF SQUARE FOR POD SET NUMBER (PN) SEED SET NUMBER (SN), SEED WEIGHT (SW) AND DAYS TO 50% FLOWERING (DF) UNDER DIFFERENT TEMPERATURE RANGE AND DIFFERENT DATES

Source of variation	DF	PSN	SN	SW	DF
Replicate	3	64.013	168.83	99.43	11
Cultivar	1	12.95	13.4	0.1	27
Temperature	1	270.03***	4810.4***	1084.5***	29
Dates	1	16.96	26.6	608.3***	4451***
Cultivar: Temperature	1	0.24	136.2	10.8	122*
Cultivar: Dates	1	57.11*	248.7	246.4*	238**
Temperature: Dates	1	1.46	384.5	90.0	77
Cultivar:Temperature:Dates	1	47.57	524.7*	135.0	266**
Residuals	116	13.52	114.04	39.25	28.58

Results indicated by *** are highly significant, * significant

APPENDIX 2.

MEAN SUMS OF SQUARE FOR PROTEIN AND SUGARS UNDER DIFFERENT TEMPERATURE RANGE AND DIFFERENT DATES

Source of variation	DF	Protein	Sugars
Replicate	3	5.82	0.191
Cultivar	1	35.20*	9.790***
Temperature	1	0.00	0.525
Dates	1	9.37	0.263
Cultivar: Temperature	1	3.09	0.195
Cultivar: Dates	1	2.29	0.340
Temperature: Dates	1	0.00	0.090
Cultivar:Temperature:Dates	1	0.04	0.053
Residuals	21	5.43	0.286

Results indicated by *** are highly significant, * significant

APPENDIX 3.

MEAN SUMS OF SQUARE FOR SEED QUALITY TRAITS UNDER DIFFERENT TEMPERATURE RANGE AND DIFFERENT DATES

Source of variation	Df	Germination (%)	SDW(g)	DRW(g)	HH (%)
Replicate	3	8.66	0.0236	0.04304	66.41
Cultivar	1	26.66	0.5420***	0.08480**	60.45
Temperature	1	15.73	0.4421***	0.20597***	102.66
Dates	1	42.55*	0.0758*	0.01962	49.95
Cultivar: Temperature	1	20.10	0.0187	0.03209	60.45
Cultivar: Dates	1	36.06*	0.0183	0.04459*	88.83
Temperature: Dates	1	9.76	0.0011	0.00065	49.95
Cultivar:Temperature:Dates	1	28.36	0.0199	0.03399	88.83
Residuals	21	8.24	0.0091	0.20937	

APPENDIX 4.

THE SEED GERMINATION OF DRY SEEDS BEFORE SOWING

Cultivar	Germination (%)	Abnormal seeds (%)	Death seeds (%)	Hollow heart (%)
Alderman	96	3	1	30.4
Greenfeast	92	6	1	2.2
Organic progress	87	5	8	6.0
Snow pea	97	1.5	1.5	1.1
Sugar snap	84.5	4.5	11	3.3
WF Massey	97	2.5	0.5	2.7

APPENDIX 5.

PROTEIN AND SOLUBLE SUGARS OF THE DRY SEEDS BEFORE SOWING

Cultivar	Protein %	Fructose % m/m	Glucose % m/m	Lactose Anydrous % m/m	Maltose % m/m	Sucrose % m/m	Galactose % m/m
Pea Greenfeast	22.1	0.2	0.4	<0.1	0.2	7.2	1.0
Pea Sugar Snap Dwarf	23.9	0.2	0.3	<0.1	0.1	6.1	0.7
Snow pea	21.2	0.2	0.3	<0.1	0.1	3.5	0.6
Organic Pea Progress	27.1	0.5	0.5	<0.1	<0.1	7.8	0.8
Pea WF Massey	24.1	0.1	0.2	<0.1	<0.1	3.6	0.7