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# Thapa, Raksha Kiran, <u>Ancient Spring Wheat Production in Wyoming: Effect of Nitrogen, Location, and Crop Type on Growth, Yield, and Quality,</u> MS, Plant Science, August 2021.

Einkorn (Triticum monococcum L.), emmer (Triticum turgidum L.), and spelt (Triticum spelta

L.) are the hulled ancient species of wheat that are currently attracting renewed consumer interest and can be potential alternative crops in Wyoming. This study was conducted to identify the agronomic potential of spring spelt, emmer, and einkorn in Wyoming. Crops were grown in three locations (Powell, Sheridan, and Lingle, WY), under dryland and irrigated conditions, and with three levels of nitrogen fertility applied. Ancient spring wheats were slower to mature than modern spring wheat with einkorn being the slowest. There was no significant effect of preplanting surface nitrogen application on the growth, yield, and quality of either ancient or modern wheats under irrigated and dryland conditions. Irrigated yields were highest in Powell and dryland yields were highest in Sheridan. Both northern locations, Powell and Sheridan, were more suitable for the production of ancient spring wheats than the southern location (Lingle). Among the ancient wheats, emmer yielded the highest across locations and appeared the most adapted for Wyoming production under both irrigated and dryland growing conditions. Future studies adapting measures to reduce nitrogen losses such as soil nitrogen incorporation, split season nitrogen application, and using additional varieties, more locations, and economic analysis will be necessary to fully understand the true potential of ancient wheat in Wyoming. Standard small grain production practices will need to be modified to accommodate differences in crop maturity and the hulled nature of the ancient spring wheats relative to modern wheat and barley currently produced in Wyoming.

# Ancient Spring Wheat Production in Wyoming: Effect of Nitrogen, Location, and Crop Type on Growth, Yield, and Quality

By

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in partial fulfillment of the requirements

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in

#### **PLANT SCIENCES**

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# DEDICATION

I would like to dedicate this thesis to my parents, Sher Bahadur Thapa and Maya Thapa, for their inspiration and wholehearted support in my career.

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#### **CHAPTER 1. LITERATURE REVIEW**

Einkorn (Triticum monococcum L.), emmer (Triticum turgidum L.), and spelt (Triticum spelta L.) are ancient species of wheat currently attracting renewed interest (Shewry & Hey, 2015). Ancient wheats are hulled wheats that were grown historically for food and feed (Hajnalová & Dreslerová, 2010; Ratajczak et al., 2020; Zaharieva et al., 2010). However, they were replaced by modern wheat (Triticum aestimum L.) because of their low yield, tall nature, and difficulty in separating the grain from the hull (Okuno et al., 2014). The grains of most ancient wheats are tightly enclosed by tough glumes and require a separate dehulling treatment to separate the chaff from the grain, though there are a few ancient wheat varieties that are free threshing (Longin et al., 2016). Ancient wheats are considered to be healthier wheat options and are a good source of proteins, lipids, fructans, trace elements, and several antioxidant compounds (Hidalgo & Brandolini, 2014; Longin et al., 2016). The increasing demand for traditional products, the need to preserve genetic diversity, and the high adaptability of ancient species in marginal environments, together with better nutritional composition than modern bread and durum (Triticum durum L.) wheat, are some of the reasons ancient wheat have had renewed attention (Troccoli & Codianni, 2005; Carnevali et al., 2014). The relative economic return associated with ancient wheats is greater than modern wheat due to their products' high prices (Cadeddu et al., 2021). The higher price offsets the lower yield of the ancient wheats maintaining profit margins.

#### 1.1. Origin and domestication of ancient grains

Einkorn was the first cultivated wheat (Zaharieva & Monneveux, 2014). It is diploid, with two sets of chromosomes (genome AA, two complements of seven chromosomes, 2n = 14) (Hidalgo & Brandolini, 2014). *Triticum b. supsp. thaoudar* is wild einkorn and the ancestor of cultivated einkorn (Brandolini & Heun, 2019). It was domesticated approximately 9000 BC, likely in Turkey (Piperno et al., 2004). It then spread to the middle-East, the Balkans and Caucasus, Turkmenistan, Central, and Mediterranean Europe, North-Africa, and Western and Northern Europe (Zaharieva & Monneveux, 2014). Today, traditional domesticated einkorn is grown in mountain areas of the Mediterranean region, and wild species still thrive in central and eastern parts of the Fertile Crescent (Hidalgo & Brandolini, 2014).

Emmer was domesticated from wild emmer (*T. turgidum ssp dicoccoides*), which evolved through the hybridization of two diploid grasses, *Triticum uratu* and an unknown grass species closely related to *Aegilops speltoides*, giving emmer the tetraploid genome, AABB (2n = 4x = 28) (Özkan et al., 2011, Arzani & Ashraf, 2017). *Triticum uartu* is the source of genomes AA, and the unknown grass is the source of genome BB (Cooper, 2015). Emmer was domesticated in a fertile crescent in 8500 BC and spread in Greece, Cyprus, India, and Egypt (Cooper, 2015). It is now a minor crop. However, countries like Yemen, India, and Ethiopia still grow and use it for making traditional foods (Zaharieva et al., 2010).

Spelt is hexaploid wheat (2n=6x=42) with genome AABBDD (Arzani & Ashraf, 2017). It is the hybrid of hulled tetraploid emmer (genome AABB) and *Aegilops tauschii* having genomes DD (Dvorak et al., 2012). Spelt was domesticated in 5000 BC in the region of Iraq and spread west (Nature's legacy, 2021). During the last three decades, spelt was grown on more than 10,000 hectares of land in Germany and neighboring countries (Rapp et al., 2017). Spelt was introduced

to the United States in 1890s (Oplinger, et al.). Spelt is the most likely ancestor of the free threshing modern bread wheat (Arzani & Ashraf, 2017).

#### 1.2. Agronomy of ancient grains

The ancient wheats, einkorn, emmer, and spelt are less domesticated than the modern wheat and have significant differences in growth character, plant height, grain yield, and maturation time compared to modern bread and durum wheats (Longin et al., 2016, Arzani & Ashraf, 2017).

#### **1.2.1.** Growth and maturity

Ancient wheats tend to grow taller than the modern wheat which can lead to problems with lodging in the field and resource distribution to biomass instead of grain production. Longin et al. (2016) found that all ancient wheats (spelt, emmer, einkorn) were 30 cm taller than modern wheat (average height of 90 cm) which may be the reason for lodging of ancient wheats but not modern wheat in their study. Castagna et al. (1996) reported that the average height of einkorn, emmer, spelt, and bread wheat were 105, 119, 113, and 69 cm respectively, with ancient wheats 36-50 cm taller than bread wheat. They observed lodging issues in einkorn and emmer, but not in spelt or modern wheat. The amount of lodging in wheat often depends on the variety grown. Konvalina et al. (2010) noticed that different varieties of ancient and modern wheat had different inclination to lodging resistance. Chapagain & Riseman (2012) observed 10% lodging in the commercial modern wheat variety 'Snowbird' and 0% lodging in five other commercial modern wheat varieties. They saw 60% lodging in the emmer variety 'Emmer-1', 0% lodging in the emmer variety 'Emmer-2', and 0% lodging in einkorn. Modern wheat variety 'Snowbird' was taller than other commercial wheat varieties that might be the reason for its lodging. However, the height of einkorn (126 cm) and 'Emmer-2' (108 cm), which did not have lodging issues, was higher than emmer variety 'Emmer-1' (105 cm). They concluded plant height and location and

direction of wind flow as factors for lodging of these varieties. Lodging in cereals can also occur due to several other reasons like high nitrogen level, high seeding rates, wet soil, and poor straw strength (Ransom, 2015). Lodging reduces the plants' ability to complete grain ripening and results in light and shriveled kernels (Troccoli & Codianni, 2005; Longin et al., 2016). Increased lodging in ancient wheats can result in poor performance of ancient wheats compared to modern wheat (Law et al., 1978).

Ancient wheats have slower time to maturity than modern wheat. Chapagain & Riseman (2012) reported that ancient wheats matured one to four weeks later than modern wheat in Vancouver, Canada. In their study, modern wheat, einkorn, and emmer took an average of 100, 125, 110 days respectively to reach harvesting stage. Troccoli & Codianni (2005) compared the days to heading among the ancient wheats and found that emmer headed earlier, followed by spelt, and then einkorn. Castagna et al. (1996) reported a similar finding in which modern bread wheat headed 10 days earlier than emmer, 16 days earlier than spelt and 24 days earlier than einkorn. Longin et al. (2016) reported that modern wheat, emmer, and spelt had similar heading time, but einkorn headed 10 days later than modern wheat. Hence, ancient wheats have longer growing season than modern wheat and replacing modern wheat by ancient wheat might require some alteration in the common crop rotation practices. Besides, the timeline of crop growth is different for ancient wheats compared to modern wheat which changes the climatic conditions during various vegetative and reproductive growth stages, current pest type and their cycles, weed species and amount of their infestation, and water and input requirements. Such changes may require farmers to change current agronomic management practices and timing of intercultural practices. Early planting might be required for ancient wheats to ensure

favorable temperature during flowering and grain filling period. Type and timing of herbicide and pesticide use may also need to be changed to combat pest and weed problems.

Ancient wheats have taller height, higher lodging, and slower maturity than modern wheat. Lodging causes problems in performing intercultural operations as well as reduces the yield by resulting in light and shriveled kernels. Complete lodging can even make the crop unable to harvest and result in 100% loss. Several factors like high nitrogen application, seeding rate, and irrigation increase lodging. Marino et al. (2011) reported that increasing nitrogen from 0-30 kg ha<sup>-1</sup> increased height of emmer. Nitrogen rate, irrigation, and seeding rate should be reduced in ancient wheat production compared to modern wheat which can potentially decrease the height and lodging issues. Few accessions having lower plant height and consequently lower lodging issues have been bred (Watanabe, 2017). More breeding efforts are necessary to get higher yielding and shorter ancient wheats. Due to the longer crop period of ancient wheats compared to modern wheat, farmers should check whether they can fit ancient wheats in their cropping system without impacting the whole crop rotation practice and any alteration in crop management and rotation practices must be made to make the maximum profit.

#### 1.2.2. Yield (hulled and grain) and yield parameters

The average yield potential and yield of ancient wheats is lower than modern wheat (Castagna et al., 1996; Longin et al., 2016). Longin et al. (2016) reported that the mean grain yield of fifteen accessions each of bread wheat, durum wheat, spelt, emmer, and einkorn were 8000, 6100, 5000, 3600, and 2700 kg ha<sup>-1</sup>, respectively in southern Germany. Similarly, Castagna et al. (1996) reported average grain yields of bread wheat 'Eridano', spelt 'Altgold Rotkorn', emmer 'Campobasso', and einkorn 'German Winterform' in their study were 3860 kg ha<sup>-1</sup>, 2710 kg ha<sup>-1</sup>, 2371 kg ha<sup>-1</sup>, and 1061 kg ha<sup>-1</sup>. Yield of ancient wheat and modern wheat can be affected by

location, wheat species, and variety of ancient wheat used (Hlisnikovský et al., 2019) and any one of these factors might be the reason for difference in grain yield observed in the two studies. Different ancient wheat may outperform another ancient wheat depending upon the variety used, soil and climatic condition of the location. Troccoli & Codianni (2005) reported a higher hulled grain yield of emmer 'Dicocco Molise' than spelt 'Altgold Rotkorn' and einkorn 'Winterform' in southern Italy. The yield of the mentioned varieties of einkorn, emmer, and spelt in their study was 3540 kg ha<sup>-1</sup>, 2800 kg ha<sup>-1</sup>, and 1420 kg ha<sup>-1</sup> respectively. Longin et al. (2016) determined the mean grain yield of bread wheat, durum wheat, spelt, emmer, and einkorn as 8000 kg ha<sup>-1</sup>, 6100 kg ha<sup>-1</sup>, 5000 kg ha<sup>-1</sup>, 3600 kg ha<sup>-1</sup>, 2700 kg ha<sup>-1</sup> respectively and reported that there was variation in yield of the varieties within the wheat species. Similarly, Castagna et al. (1995) compared the hulled yield of einkorn by location and found the highest hulled yield of 4500 kg ha<sup>-1</sup> in Cologne, Germany, and the lowest 840 kg ha<sup>-1</sup> in Foggia, Italy.

Short, dense spikes have a negative effect on grain yield, as the spike density is negatively correlated with the weight of thousand grains (TGW), the weight of the grains in the spikes, the number of grains in the spikelets, and the proportion of hulls to the weight of the grains (Konvalina et al., 2011). Einkorn and emmer wheat have short and dense spikes and a low thousand grain weights, whereas spelt wheat has long and lax spikes (Figure 1). This might be one reason for the higher yield of spelt than emmer and einkorn (Konvalina et al., 2011). Ancient wheat yield can be increased by selecting cultivars having long, lax spikes and a high thousand grain weight (Konvalina et al., 2011).



### Figure 1.1. Spike of Ancient Wheats

Figure 1.1. Spike of ancient wheats A) Short and dense einkorn spike B) Long and lax spelt spike C) Short and dense emmer spike

#### 1.2.3. Agronomic management practices

Most of the agronomic management practices for ancient wheats are similar to modern wheat. However, some unique characteristics of ancient wheats, as mentioned above, require alteration of some management practices. Ancient wheats are hulled, their grains are enclosed by tough, thick, tenacious glumes, and the grain does not thresh free during harvest (Kerber & Rowland, 1974). Ancient wheat requires a special dehulling treatment before milling to separate the chaff from the grain (Longin et al., 2016).

Hulled seed is commonly used to plant ancient wheats whereas naked seed is used to plant modern wheat (Dorval et al., 2015). Using the hulled seeds while planting can cause problems like clogging in the drill seeder (Dorval et al., 2015). However, planting hulled seeds of ancient wheats is preferred, as the dehulling process can affect grain integrity and germination rate (Dorval et al., 2015). Each ancient wheat has a unique hull size and thousand seed weight

(Figure 2). So, the appropriate seeding rate (seeds  $m^{-2}$ ) to get maximum yield can vary for each wheat species. Troccoli & Codianni (2005) reported that the hulled grain yield of emmer and spelt increased with increasing seeding rate whereas that of einkorn decreased with increasing seeding rate from 100 seeds m<sup>-2</sup> to 200 seeds m<sup>-2</sup> in southern Italy. The yield of spelt, emmer, and einkorn at 200 seeds m<sup>-2</sup> was 3090 kg ha<sup>-1</sup>, 3850 kg ha<sup>-1</sup>, and 1130 kg ha<sup>-1</sup> respectively whereas the yield of spelt, emmer, einkorn at 100 seeds m<sup>-2</sup> was 2750 kg ha<sup>-1</sup>, 3250 kg ha<sup>-1</sup>, and 1690 kg ha<sup>-1</sup> respectively in their study. Castagna et al. (1995) found a similar result in which increasing seeding rate of 21 einkorn lines from 100-600 seeds m<sup>-2</sup> did not increase yield in Germany or Italy. However, Castagna et al. (1996) found a further increase in yield of all ancient wheats, spelt, emmer, einkorn, and modern wheat on increasing seeding rate from 200 to 400 seeds m<sup>-2</sup> in Italy. The higher spelt and emmer hulled yields associated with higher sowing rates are mostly due to the increase in spike number per unit area, followed by kernels per spike, and secondarily, by kernel weight (Donaldson et al., 2001; Tompkins et al., 1991). Castagna et al. (1995) suggested that einkorn cannot tolerate denser spike populations which may be the reason for lower seeding rate requirement of einkorn.

Ancient wheat species have historically been cultivated under low-input conditions. They are believed to have high nitrogen use efficiency and perform better than modern wheat at low nitrogen application rates. Fatholahi et al. (2020) found that yield of modern wheats increased on increasing nitrogen from 0-75 kg ha<sup>-1</sup> whereas yield of ancient wheat was similar in all nitrogen treatments. The protein content of both modern wheat and ancient wheats increased with increasing nitrogen from 0-120 kg ha<sup>-1</sup> and 0-80 kg ha<sup>-1</sup> respectively in their study. Castagna et al. (1996) had a similar finding in which increasing nitrogen from 0 kg ha<sup>-1</sup> to 50 kg ha<sup>-1</sup> and 100 kg ha<sup>-1</sup> did not increase the yield of all three ancient wheats. However, there was significant

increase in grain yield of modern wheat on increasing nitrogen from 0 kg ha<sup>-1</sup> to 50 kg ha<sup>-1</sup> and then yield remained similar in 50 kg ha<sup>-1</sup> and 100 kg ha<sup>-1</sup> nitrogen application in their study. Similar to Fatholahi's findings, increasing nitrogen increased protein content of all ancient wheat and modern wheat in Castagna's study. Castagna et al. (1995) also reported that all einkorn lines used in their study were unresponsive to nitrogen for grain yield and plant height. The three levels of nitrogen (0, 80, and 120 kg ha) didn't influence grain yield or plant height in their study. Vaghar & Ehsanzadeh (2018) also observed that emmer lines were unresponsive to nitrogen. Alemu (2016) even reported the highest emmer yield at 0-23 kg ha<sup>-1</sup> compared to a higher nitrogen application (46 kg ha<sup>-1</sup> and 69 kg ha<sup>-1</sup>). Ancient wheats seem to have smaller nitrogen uptake, utilization, remobilization, use efficiency, grain yield, and harvest index compared to the improved durum and bread wheats, particularly in the presence of sufficient/higher nitrogen supplies (Fatholahi et al., 2020). The above studies support that the nitrogen requirement of ancient wheats is lower than the modern wheat. However, there are contrasting results reported in other studies. Marino et al. (2016) reported that emmer had both higher yield and protein content at 90 kg ha<sup>-1</sup> than 0 kg ha<sup>-1</sup> nitrogen application. Marino et al. (2011) also found that increasing nitrogen rates from 0 to 30 to 60 and further to 90 kg  $ha^{-1}$ increased hulled yield, net grain yield, and protein content of emmer. Due to contrasting results found so far, nitrogen demand of ancient wheats is still unknown and more regionally specific studies with different rates of nitrogen is suggested.

Timing of nitrogen application is crucial in obtaining high yields in modern wheat (Lopez-Bellido, Fuentes, Castillo, Lopez-Garrido, & Fernandez, 1996). Nitrogen splitting at vegetative growth stages like tillering and jointing may reduce nitrogen losses and lead to a better translocation of pre-anthesis assimilates to the grain (Abdin et al., 1996). Nitrogen splitting may

also be useful in ancient wheat production. Castagna et al. (1996) made a split nitrogen application and found that the protein content increased on increasing nitrogen but the grain yield remained constant. Marino et al. (2011) reported that nitrogen application at tillering increased net grain yield of emmer by 36% in comparison to nitrogen application at seeding and stem elongation stage and that emmer had highest grain protein yield per hectare when split application of 30-30-30 kg ha<sup>-1</sup> was applied at seeding, tillering and stem elongation stages in their study.

Like modern wheat, ancient wheats require weed management for optimum yield. Each ancient wheat species has a different competitive ability and weed management may need to be adjusted. Troccoli et al. (1997) reported that most of the yield and quality parameters of ancient wheats increased with weed control by using diclofop-methyl, at the rate of 568 a.i./ha. He created three artificial weed levels (0%, 50%, and 100% of seeding rates used for ancient wheats) with *Avena fatua* and *Phalaris arundinacea* and noticed that yield loss to weed infestation vs chemical weed control was higher for einkorn and spelt than emmer. This suggests that emmer has more weed tolerance capacity than einkorn and spelt. However, more regionally specific studies and using different artificial weeds is needed to determine the weed tolerance capacity of the ancient wheats.

Hulls have been found to protect the ancient grain seeds from soil-borne fungal diseases (Riesen et al., 1986) and limit toxin accumulation associated with diseases like fusarium head blight (Vučković et al., 2013). Hence, ancient wheats require fewer pesticides before sowing than modern wheat (Escarnot et al., 2010; Koenig et al., 2015). Ancient wheats also have resistance to certain diseases that are common to modern wheat. The disease resistance varies by the ancient wheat species. Konvalina et al. (2010) reported that einkorn and emmer were resistant to

powdery mildew and brown rust, whereas spelt wheat was less resistant to these two diseases. Einkorn cultivar RL 5244 is resistant to stem rust in western Canada (Kerber & Dyck, 1973). Ouyang et al. (2014) and Hua et al. (2009) identified powdery mildew resistant genes in wild emmer accession. But these resistances vary largely by varieties/accessions of the ancient wheats. The ancient wheat varieties having such resistance traits may require fewer pesticide applications compared to modern wheat, making them agronomically advantageous and reducing cost. Several efforts have also been made to transfer resistance from ancient wheats to modern wheat.

The ancient wheats are also believed to have high water use efficiency and suitability for marginal and organic farming (Vaghar & Ehsanzadeh, 2018). Konvalina et al. (2012) reported that all emmer accessions used in their study had higher resistance to drought than the modern wheat. Cadeddu et al. (2021) reported that grain yield from the einkorn and emmer used for dual purpose (grain + forage) in the same season under water stress was comparable to average yield under well-watered conditions and without forage harvest.

Vaghar & Ehsanzadeh (2018) reported that modern wheat had severe water stress symptoms on 30-40% and 60-70% of depletion of available soil water whereas emmer showed minimal modification of photosynthetic pigments on water stress. But, intrinsic water use efficiency (WUE<sub>i</sub>) of modern wheat and emmer increased and decreased respectively under 100 kg ha<sup>-1</sup> nitrogen application vs 30 kg ha<sup>-1</sup> nitrogen application in their study. Khazaei et al. (2009) found that modern bread wheat had higher water use efficiency for grain than einkorn under well-watered conditions. This suggests that ancient wheats might require less water and can tolerate drought, but their water use efficiency for grain yield decreases with increased nitrogen.

**1.3.** Breeding of ancient wheats and use of ancient wheat in modern wheat breeding Ancient wheats have several drawbacks like lower yield, non-free threshing habit, late maturity, taller heights, and lodging which can be improved through breeding efforts. Several efforts have been made to improve the agronomic performance of ancient wheats. Watanabe (2017) found that the sog gene and sos gene linked with the gene for semi-dwarfism from Triticum sinskajae govern the free threshing trait of einkorn. They also developed semi-dwarf lines and early freethreshing lines using genes from various sources and then crossed them to obtain early, free threshing semi-dwarf pre-breeding einkorn germplasm. These pre-breeding materials can be further used to produce modern high yielding, free threshing, early maturing and dwarf einkorn varieties. Konvalina et al. (2011) studied various emmer accessions to find a way to increase productivity and observed that the productivity of emmer can be increased by selecting cultivars having long lax spikes and higher test weight. Packa et al. (2019) identified spelt breeding lines with high yield components, high grain quality and lower sensitivity to lodging and suggested that those breeding lines can further be used for the improvement of spelt cultivars. Studies have also been carried out to improve bread making quality of ancient wheats while maintaining nutritional properties. Sobczyk et al. (2017) reported that the new spelt breeding line 'STH8' had good baking parameters similar to modern wheat. There is high genetic diversity even within the ancient wheat species. There are several landraces and wild accessions of ancient wheat species that differ by yield, nutrient composition, baking performance, growth habit, and several other beneficial traits (Bencze et al., 2020). Such landraces can be used to further improve the registered varieties of ancient wheat species.

Ancient wheats can also be used in modern wheat improvement programs. Some studies have been done to identify and transfer disease resistant genes from accessions of ancient wheats to

modern wheat. Ouyang et al. (2014) identified a powdery mildew resistant gene 'MIIW172' 'in wild emmer accession 'IW172'. Hua et al. (2009) backcrossed susceptible common wheat with wild emmer accession 'G-303-1M' having powdery mildew resistance and obtained a powdery mildew resistance common wheat line 'P63'. He also found that powdery mildew resistance in common wheat line 'P63' was controlled be single recessive gene 'pm42'. Similarly, Kerber & Dyck (1973) transferred a stem rust resistance gene from einkorn cultivar 'RL 5244', to durum wheat and common wheat by interspecific hybridization. Both cultivated and wild ancient wheat accessions have several beneficial traits, which can be used in a modern wheat breeding program. Improving nutritional and health benefits of modern wheat by using ancient wheats can be a future goal of modern wheat breeding.

#### 1.4. Value addition of ancient wheats

Chaff obtained from ancient wheats can be used for various purposes that can add to the total profit obtained from these ancient wheats and offset yield losses. Hulled wheat chaff can be a cheaper alternative energy source than lignite in the region of cultivation and processing (Bernas et al., 2020; Wiwart et al., 2017). Hulls can also be composted, used as litter or additive to building materials, or could be directly put back to the agricultural land to maintain soil fertility (Bernas et al., 2020; Wiwart et al., 2017). Ancient wheats can also be used for dual purpose to produce forage and grain in the same season. Cadeddu et al. (2021) clipped the above ground biomass (herbage) from einkorn and emmer early in the season to feed animals and found no yield decline at harvest. Using chaff as an energy source and compost feedstock and harvesting green forage from ancient wheats as animal feed can add to the profit obtained from growing ancient wheats.

#### 1.5. Human uses and nutritional benefits of ancient wheats

There has been a renewed interest of consumers in the unique nutritional composition, flavor, and health benefits of ancient wheats (Longin et al., 2016). Ancient wheats have a higher amount of protein, minerals, and bioactive phytochemicals (carotenoids, tocopherols) than modern wheat and are suitable for producing high-value food products (Shewry & Hey, 2015). Ancient wheats were grown historically for food and feed (Hajnalová & Dreslerová, 2010; Ratajczak et al., 2020; Zaharieva et al., 2010). Einkorn and emmer were used to feed pigs, chickens, and horses (Hajnalová & Dreslerová, 2010; Zaharieva et al., 2010). Their straw was used in the construction of thatched roofs of primitive houses (Padulosi & Hammer, 1995). Spelt, emmer, and einkorn were also used to make leavened/unleavened bread, porridge, gruel, soup, cracked grains, and beer for human consumption (Nesbitt & Samuel, 1996; Braun, 1995). However, modern bread wheat and durum wheat replaced them for several reasons. Free-threshing habit, higher yielding, wider adaptability to different agroclimatic conditions, and better baking qualities of modern wheat compared to ancient wheats are some of reasons for their replacement (Arzani & Ashraf, 2017; Ratajczak et al., 2020). Because of the increasing consumer interest, einkorn, emmer and spelt based products are now available in the market, including flour, bread, breakfast cereals, pasta, and crackers (Mayer et al., 2011; Buerli, 2006). Emmer and spelt are also used for making beer (Fujita et al., 2020). Benedetti et al. (2016) used the emmer malt to produce a light beer, a double malt beer, and beers with emmer malt combined with barley malt. Emmer grain is also used in traditional soups in Tuscany (Zaharieva et al., 2010).

#### 1.5.1. Protein composition and bread making potential of ancient wheats

The protein content is higher in ancient wheats than modern bread wheat (Longin et al. 2016; Table 1). Each ancient wheat has unique protein content and composition. The average protein content is the highest in spelt (13.3%), followed by einkorn (13.3%) emmer (12.8%), and bread wheat (12.3%) (Table 1). But Hidalgo & Brandolini (2014) performed a survey of 65 different einkorn species and reported an average protein content of 18.2%, which is higher than reported by USDA (Table 1). Further research into the effect of environment and accessions of ancient wheats is required to fully understand the protein content potential of ancient wheat species. Wheat is used to make baked food products. Good baking quality of the ancient wheats is essential to replace modern wheat in food products. The baking quality is complex and determined both by total grain protein and composition/quality (Chaudhary et al., 2016). High total protein content is not of value if the protein quality is poor (Longin et al., 2016). Wheat protein has two major gluten (glutenin and gliadin) and nongluten (globulin and albumin) fractions (Arzani & Ashraf, 2017). Glutenin can impart strength and elasticity to dough, whereas the gliadins are responsible for dough viscosity (D'Ovidio & Masci, 2004). Protein quality is determined by the glutenin: gliadin ratio in the gluten. Higher glutenin content and a lower gliadin:glutenin ratio leads to higher baking volume (rise) and better overall baking performance (Geisslitz et al., 2018).

Modern bread wheat has a lower gliadin:glutenin ratio than the ancient wheats and forms a viscoelastic dough with a high gas holding capacity when it is mixed with water (Geisslitz et al., 2018) making common bread wheat flour suitable for bread making. In contrast, ancient wheats have higher gliadin:glutenin ratios, and their flours make softer dough with low elasticity and high extensibility (Sobczyk et al., 2017; Wieser et al., 2009). Geisslitz et al. (2018) reported a

similar finding where ancient wheats had higher gluten content and higher gliadin:glutenin ratios than modern bread wheat in all 4 Germany locations. Gliadin:glutenin ratios ranged from einkorn (<12.2:1), emmer (< 11:1), spelt (< 5:1) to common wheat (< 3.8:1). Similarly, Wieser et al. (2009) reported that gliadin:glutenin ratios were higher in einkorn compared to common wheat. However, Abdel-Aal et al. (1995) reported that the gliadin:glutenin ratios were 2:1 for einkorn; 1:1 for spelt SK0021 and spelt PGR8801 and common hard red spring wheat; and 0.8:1 for durum and spelt SK0505, spelt SK0263, and RL5407 wheat flour proteins. These differences are due to location and variety used (Longin et al., 2016; Rapp et al., 2017). Ancient wheat accessions with lower gliadin:glutenin ratios may be more suitable for baking and efforts to identify accessions of ancient wheats with lower gliadin:glutenin ratios are ongoing.

Borghi (1996) identified approximately 16% of the total einkorn accessions with good breadmaking potential. Doughs prepared from ancient wheats are usually sticky, difficult to handle, have lower stability, less elasticity, and higher extensibility than bread wheat that can cause difficulty in making bread (Boukid et al., 2018; Frakolaki et al., 2018). Even with improved protein content, making bread from ancient wheats might require modified baking techniques like the addition of ascorbic acid, decreasing mixing times and amount of water added, or elongating of dough rest times (Frakolaki et al., 2018).

#### 1.5.2. Nutrient composition

Ancient wheats provide higher total energy (kcal/100 g) than modern wheat (Table 1.1). They provide higher carbohydrates, protein, dietary fiber, and zinc than modern wheat (Table 1.1) making them suitable for high value food products.

#### Table 1.1. Chemical Composition of Wheat Species

Chemical composition	Einkorn	Emmer	Spelt	Modern Bread Wheat
Energy (kcal/100 g)	333.0	362.0	378.0	254.0
Carbohydrate (g/100)	67.0	72.3	73.3	43.1
Protein (g/100g)	13.3	12.8	13.3	12.3
Dietary Fiber (g/100g)	6.7	10.6	15.6	6.0
Lipid (g/100g)	1.7	2.1	2.2	3.6
Calcium (mg/100g)	0	Nr <sup>1</sup>	24.0	163.0
Iron (mg/100g)	3.6	1.5	2.2	2.6
Magnesium (mg/100g)	2.0	128.0	Nr	76.6
Zinc(mg/100g)	15.0	4.8	Nr	1.7

Table 1.1. Chemical composition of ancient wheat in terms of macro and micronutrients (USDA,2018, 2019a, 2019b, 2021).

 $^{1}$ Nr = Not reported.

#### 1.6. Conclusion

Einkorn, emmer, and spelt are the hulled ancient species of wheat that are currently attracting renewed interest (Shewry & Hey, 2015). They are good sources of proteins, lipids, fructans, trace elements, and several antioxidant compounds (Hidalgo & Brandolini, 2014; Longin et al., 2016). They are believed to have high nitrogen use efficiency and perform well even with limited resources (Pourazari et al., 2015). Historically, ancient grains were grown on marginal lands for food and feed (Hajnalová & Dreslerová, 2010; Ratajczak et al., 2020; Zaharieva et al., 2010) but, they were replaced by modern wheat because of their low yield, excessive tall nature, and difficulty separating the grain from the hull (Okuno et al., 2014). The increasing demand for traditional products, the need to preserve genetic diversity, and the high adaptability of ancient species in marginal environments together with better nutritional composition than modern bread and durum wheat are among the reasons behind the renewed attention toward ancient wheats (Troccoli & Codianni, 2005; Carnevali et al., 2014).

The ancient wheats are less domesticated than the modern wheat (Arzani & Ashraf, 2017). Thus, there is a significant difference in grain yield, plant height, and maturity time of these species compared to modern bread and durum wheat (Longin et al., 2016). Growth, agronomic performance, maturity, and nutritional composition of ancient wheats vary within the species and landraces within the species (Shewry & Hey, 2015; Troccoli & Codianni, 2005; Longin et al., 2016). Management practices are different for each ancient wheat and may require some changes in the intercultural operations and management practices common to modern wheat in different growing regions. The growth and yield of ancient wheats is also affected by soil condition, climate, and variety of ancient wheat used (Hlisnikovský et al., 2019). Research on production of spelt, emmer, and einkorn in individual locations is needed to determine the appropriate management practices for these ancient wheats.

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# CHAPTER 2. ANCIENT WHEAT PRODUCTION IN WY UNDER IRRIGATION

#### 2.1. Introduction

The state of Wyoming is a challenging place to farm because of adverse soil, climatic, and geographical conditions defined by hot dry summers, wet winters, low soil fertility and quality, arid conditions, and isolation from the markets. Such conditions have historically limited crop diversity. Small grains are one of the widely grown crops in Wyoming (USDA, 2020). Wyoming farmers grew 802 acres of irrigated spring wheat and 48,895 acres of irrigated barley in 2017 (USDA, 2019).

However, consumer preferences are changing from the modern small grains used in baking and malting to ancient wheats. Einkorn (*Triticum monococcum* L.), emmer (*Triticum turgidum* L.), and spelt (*Triticum spelta* L.) are the ancient species of wheat that are currently attracting renewed interest because of their unique taste, flavor and high nutritional properties including high protein content (Shewry & Hey, 2015). Unfortunately, these wheats have lower yield and need an additional dehulling process to get the naked grain compared to the modern wheat making them more problematic to grow for farmers. However the relative economic return associated with ancient wheats can be greater than modern wheat at present due to high market demand and prices (Cadeddu et al., 2021). Ancient wheats should be well suited to Wyoming based on the success of other small grains. Considering the higher market price and demand of the ancient wheat products, ancient wheats could be profitable and potential alternative crop in irrigated fields of Wyoming.

To successfully grow ancient wheats, farmers should know the growing pattern of ancient wheats, input requirements, and appropriate farming practices required for ancient wheats. The ancient wheats are less modified than modern wheat (Arzani & Ashraf, 2017), and there can be significant differences in plant height, maturity time, and agronomic performance of these species compared to modern bread wheat (Longin et al., 2016). Longin et al. (2016) found that all ancient wheats were 30 cm taller than modern wheat (120 cm vs 90 cm). Castagna et al. (1996) reported that average height of einkorn, emmer, spelt, and bread wheat were 105, 119, 113, and 69 cm respectively where the difference in height among ancient and modern wheat is even higher than 30 cm. Lodging in cereals occurs due to several reasons like high nitrogen level, high seeding rates, wet soil, tall plant type, and varieties with poor straw (Ransom, 2015). Ancient wheats tend to have higher lodging issues than modern wheat which increases under irrigation and with high nitrogen. Longin et al. (2016) observed lodging issues in einkorn, emmer, and spelt, but not in the modern wheat even under low nitrogen application compared to modern wheat. Stallknecht, Gilbertson, & Ranney, (1996) found that einkorn and emmer had moderate straw strength and suggested that they can lodge under high moisture environments. Lodging in ancient wheat can be reduced by selecting varieties with short stems, reducing N application at sowing, and reducing irrigation during tillering (Stallknecht, Gilbertson, & Ranney, 1996; Peake, Poole, Gardner, Bell, & Das, 2017). Ancient wheats also have slower maturation than modern wheat. Troccoli & Codianni (2005) compared the days to heading among the ancient wheats and found that emmer headed earlier, followed by spelt, and then einkorn. Castagna et al. (1996) reported a similar finding in which modern bread wheat headed 10 days earlier than emmer, 16 days earlier than spelt and 24 days earlier than einkorn. Height,

lodging, and maturity differences of ancient grains will require unique management practices by growers in Wyoming.

Yield of ancient wheats vary between species and is lower than modern wheat (Castagna et al., 1996; Longin et al., 2016). Longin et al. (2016) reported that the mean grain yield of 15 accessions each of bread wheat, spelt, emmer, and einkorn were 8000, 5000, 3600, and 2700 kg ha<sup>-1</sup>, respectively in southern Germany. Castagna et al. (1996) reported a lesser grain yield but similar pattern with the highest yield of modern wheat, followed by spelt, then emmer and then einkorn in north and central areas of Italy. Average grain yields of bread wheat, spelt, emmer, and einkorn in their study were 3860 kg ha<sup>-1</sup>, 2710 kg ha<sup>-1</sup>, 2371 kg ha<sup>-1</sup>, and 1061 kg ha<sup>-1</sup> respectively. Yield of ancient wheat and modern wheat can be affected by location, wheat species, and variety of ancient wheat used (Hlisnikovský et al., 2019) and one of these factors might be reason for difference in grain yield in above two studies.

Ancient wheat species have historically been cultivated under low-input conditions. They are believed to have high nitrogen use efficiency and perform better even at low nitrogen application rate. Fatholahi et al. (2020) found that yield of modern wheats increased on increasing nitrogen from 0-75 kg ha<sup>-1</sup> whereas yield of ancient wheat was similar in all nitrogen treatments. Similarly, the protein content of modern wheat and ancient wheats increased with increasing nitrogen from 0-120 kg ha<sup>-1</sup> and 0-80 kg ha<sup>-1</sup> respectively in their study. Castagna et al. (1996) had a similar finding in which increasing nitrogen from 0 kg ha<sup>-1</sup> to 50 kg ha<sup>-1</sup> and 100 kg ha<sup>-1</sup> did not increase the yield of all three ancient wheats. However, there was significant increase in grain yield of modern wheat on increasing nitrogen from 0 kg ha<sup>-1</sup> to 50 kg ha<sup>-1</sup> and then yield remained similar in 50 kg ha<sup>-1</sup> and 100 kg ha<sup>-1</sup> nitrogen application in their study. These studies

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indicate that additional nitrogen is used to increase both yield and protein in modern wheat whereas only protein is increased in ancient wheats and that nitrogen requirement of ancient wheats is lower than the modern wheat. However, Marino et al. (2016) reported that emmer had both higher yield and protein content at 90 kg ha<sup>-1</sup> than 0 kg ha<sup>-1</sup> nitrogen application. Due to contrasting results found so far, nitrogen demand of ancient wheats is still unknown and more regionally specific studies with different rates of nitrogen are needed.

Ancient wheat should be well suited to Wyoming based on the success of other small grains. Lower yield of ancient wheats compared to modern wheat should be offset by premium markets and higher price than modern wheat. Hence ancient wheats can be potential alternative crop in Wyoming. Ancient wheats will likely have different growth characteristics and management needs than modern wheat. Their performance may also vary by growing location in WY, crop type, and management practices. In spring 2019 and 2020, we grew ancient wheats together with commonly grown modern grain in irrigated fields of three different growing regions of Wyoming under different nitrogen treatments. The objective of this study is to identify agronomic management practices and nitrogen demand of spelt, emmer, and einkorn and how nitrogen affects agronomic traits of these ancient wheats under multiple Wyoming growing conditions and locations. Our research questions are;

- Which ancient wheat is best suited for Wyoming growing conditions?
- Do ancient wheats perform differently in different growing regions?
- Are ancient grains able to maintain yield and quality under low N treatments?

## 2.2. Materials and methods

#### 2.2.1. Study site

This study was conducted in spring 2019 and 2020 at three University of Wyoming research stations; The James C. Hageman Sustainable Agriculture Research and Extension Center (SAREC) in Lingle, WY, the Sheridan Research and Extension Center (ShREC) in Sheridan WY, and the Powell Research and Extension Center (PREC) in Powell, WY (Table 2.1). Fields were irrigated with flood irrigation at PREC and overhead sprinkler irrigation at SAREC and ShREC. The average monthly temperature and total monthly precipitation for the growing season are shown in Figure 2.1 and Figure 2.2 respectively. The average monthly temperature was similar in all three stations (Figure 2.1). At SAREC, the soil textural class was loamy at 0-30 cm depth and sandy loam at 30-150 cm depth (Soil survey staff, 2021). At ShREC, the soil textural class was the mixture of loam and clay loam (Soil survey staff, 2021). At ShREC, the soil textural class was a mixture of clay loam and silt clay loam (Soil survey staff, 2021). Soil properties are described in Table 2.2.

## Table 2.1. Coordinates, Elevation, and Nitrogen Treatments of Irrigated Sites

Table 2.1. Coordinates, elevation, and nitrogen treatments at three study sites, James C. Hageman Sustainable Agriculture Research and Extension Center (SAREC), Lingle, WY, Sheridan Research and Extension Center (ShREC), Sheridan, WY, and Powell Research and Extension Center (PREC), Powell, WY in spring 2019, and spring 2020. Coordinates and elevation were obtained from google earth. Nitrogen treatments were the sum of residual soil nitrogen and applied soil nitrogen.

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Location	Coordinates	Elevation	Nitrogen Treatments					
			High N	Medium N	Low N			
		М		Kg ha <sup>-1</sup>				
SAREC	42.12 N, 104.39 W	1,272	123	90	56			
ShREC	44.76 N, 106.94 W	1,174	90	56	28			
PREC	44.90 N,108.78 W	1,331	90	56	28			



Figure 2.1. Mean Monthly Temperature (°C) in Irrigated Sites

Figure 2.1. Mean monthly temperature (°C) from planting to harvesting in spring 2019, and spring 2020 at three study sites, SAREC, Lingle, WY, ShREC, Sheridan, WY, and PREC, Powell, WY. Data was acquired from NOAA National Center for Environmental information and on-site weather station at SAREC, Lingle, WY.



Figure 2.2. Total Monthly Precipitation (mm) in Irrigated Sites

Figure 2.2. Total monthly precipitation (mm) from planting to harvesting in spring 2019, and spring 2020 at three study sites, SAREC, Lingle, WY, ShREC, Sheridan, WY, and PREC, Powell, WY. Data was acquired from NOAA National Center for Environmental information and on-site weather station at SAREC, Lingle, WY.

## Table 2.2. Soil properties

Coil Duo e ortigo	SAI	ShF	ShREC		PREC	
Soli Properties	2019	2020	2019	2020	2019	2020
рН	8.3	8.1	8.0	7.6	8.2	8.1
Organic Matter (%)	1.7	2.1	3.1	3.7	1.5	1.6
Phosphorus mg kg <sup>-1</sup> )	107	112	79	75	130	149
Potassium (kg ha <sup>-1</sup> )	453	634	177	194	196	268
Calcium (mg kg <sup>-1</sup> )	3468	3531	2904	2993	3743	4159
Magnesium (mg kg <sup>-1</sup> )	402	414	897	832	573	627
Cation Exchange Capacity [meq (100g) <sup>-1</sup> ]	24.3	23.3	22.4	22.4	24.0	26.7

Table 2.2. Chemical properties of soil at three study sites, SAREC, Lingle, WY, ShREC, Sheridan, WY, and PREC, Powell, WY in spring 2019, and spring 2020.

## 2.2.2. Field preparation, field design and planting

At PREC, plots were plowed, roller harrowed, bedded up, then planted. At ShREC, plots were direct seeded into cover crop residue. At SAREC, plots were disked and rototilled before planting. Both SAREC and PREC field were previously planted to sugar beet. A split plot design was used at each site of the experiment (Figure 3, Table 2.3). The ancient wheats, einkorn (*Triticum monococcum* L.), emmer (*Triticum turgidum* L.), spelt (*Triticum spelta* L.), and common bread wheat (*Triticum aestivum*; SHREC and SAREC) or barley (*Hordeum Vulgare*: PREC) were grown (Table 2.4). The four crops were planted on the same date within each site, but harvest dates varied with crop type (Table 2.5). All the crop species were sown at a seeding rate of 112 kg ha<sup>-1</sup> and a seeding depth of 3.8 cm. On July 9<sup>th</sup>, 2019 there was hail damage in the plots at SAREC. Plot hail damage was assessed by measuring the number of heads damaged per meter row in each plot (Klein, 2021; appendix 1).



Figure 2.3. Sample Layout of Irrigated Fields

Figure 2.3. Sample layout of irrigated fields at three study sites, SAREC, Lingle, WY, PREC, Powell, WY, and ShREC, Sheridan, WY in spring 2019 and 2020. A split plot design was used at each site of the experiment. There were three replications in each site (orange, blue, and green blocks). Each replication had 3 blocks, one for each nitrogen treatment (Table 2.1), giving a total of nine blocks in each site year. Nitrogen blocks were randomly allocated within each replication and was treated as the main plot. Crops (einkorn, emmer, spelt, and wheat/barley) were randomized in subplots within the main plot.

# Table 2.3. Subplots sizes in Irrigated Sites

PREC, Powell, WY, and ShREC, Sheridan, WY in spring 2019 and 2020.									
Locations	2019				2020				
	Length	Width	Area	Length	Width	Area			
	m	1	$m^2$	1	n	-m <sup>2</sup> -			
SAREC	10.7	4.6	49.2	12.2	1.5	18.5			
ShREC	6.1	6.1	37.2	6.1	6.1	37.2			
PREC	6.7	6.4	42.9	9.1	3.7	33.7			

Table 2.3. Plot length, width, and area of each subplot at three study sites, SAREC, Lingle, WY, PREC, Powell, WY, and ShREC, Sheridan, WY in spring 2019 and 2020.

# Table 2.4. Crop Varieties in Irrigated Sites

Table 2.4. Characteristics of the spelt, emmer, einkorn, wheat and barley varieties gown at three study site	s,
SAREC, Lingle, WY, PREC, Powell, WY, and ShREC, Sheridan, WY in spring 2019 and 2020.	

Crop	Variety	Location	Years grown	Developing company	Maturity	Disease resistance
Spelt	CDC 'Origin'	SAREC, ShREC, PREC	2019 and 2020	University of Saskatchewan (Government of Canada, 2021)	Matures in 105- 110 days	Highly resistant to loose smut and common bunt (French's hybrids, 2021)
Emmer	'Lucile'	SAREC, ShREC, PREC	2019 and 2020	Montana Foundation Seed Program (Montana Foundation Seed Program, 2003)	Heading in around 3 months (PennState, 2021)	Disease resistance is unknown (Stallknecht, 2021)
Einkorn	'Stone Age'	SAREC, ShREC, PREC	2019 and 2020	Purchased from Joel and James Starr Partnership out of Hastings, NE	It has facultative growth (spring/fall planted) (Quail seeds, 2021)	ND
Wheat	SY605 CL	SAREC	2019	Syngenta Seeds, Inc.	55 days to reach heading (WSCIA.CO, 2021).	Moderate resistance to prevalent races of leaf rust (WSCIA.CO, 2021)
	Gunnison	ShREC	2019	Westbred	Takes medium time to mature among wheat varieties	good resistance to current races of stripe rust (WSCIA.CO, 2021)
	Surpass HRSW	SAREC	2020	North Dakota State University (NDSU)	ND	Fusarium head blight and bacterial leaf streak resistance (NDSU 2016)
	Fortuna	ShREC	2020	NDSU (Heo, et al., 2018).	Medium maturity (MSU, 2021)	Resistant to prevalent races of leaf and steam rust (WSU, 2021)
Barley	Moravian 170	PREC	2019	Bob Brunick of Molson Coors Beverage Company	Early maturing (Connell, 2020)	ND
_	Miller Coors BC100	PREC	2020	Coors	Early maturing (Stamp seeds, 2021)	ND

<sup>1</sup>ND: No data

#### Table 2.5. Planting Date and Harvest Date in Irrigated Sites

Table 2.5. Planting date and harvest date of einkorn, emmer, spelt, and control at three study sites, SAREC, Lingle, WY, PREC, Powell, WY, and ShREC, Sheridan, WY in spring 2019, and spring 2020. Wheat was used as a control grain at SAREC and ShREC and barley was used as a control grain at PREC. At ShREC, plots were lost in both years due to bird damage.

Location	Plan Da	iting ate		Harvest Date							
				201	9				202	20	
	2019	2020	Einkorn	Emmer	Spelt	Control		Einkorn	Emmer	Spelt	Control
PREC	4-16	3-31	9-19	8-27	8-27	8-21	-	8-24	8-14	8-14	8-14
SAREC	5-06	4-7	9-9	8-23	8-27	8-18		8-26	7-28	8-26	7-24
ShREC	5-18	4-22	$ND^1$	ND	ND	ND		ND	ND	ND	ND

<sup>1</sup>ND means no data.

## 2.2.3. Soil sampling and nitrogen application

At PREC, and SAREC, soil was sampled pe-plant and post-harvest in each plot at three depths (0-20 cm, 20-60 cm, 60-90 cm). At ShREC, soil sampling was done before planting and composite samples for the study area were taken at all three depths. Soil samples were analyzed at Midwest laboratories Inc., Ohama, NE. Pre-planting residual soil nitrogen at 0-20 cm soil depth was calculated. In PREC and SAREC, the average residual soil nitrogen in each block was calculated using eqn (1) and at ShREC it was calculated using eqn (2). 28, 56, 90 kg ha<sup>-1</sup> were assigned as high, medium, and low N treatments in ShREC and PREC (Table 2.1). The residual soil nitrogen at SAREC was higher than at the other two locations and exceeded 28 kg ha<sup>-1</sup> so, the nitrogen treatments rates at SAREC were increased to 123, 90, 56 kg ha<sup>-1</sup> as high, medium, and low nitrogen treatments respectively (Table 2.1). Nitrogen treatments were the sum of the residual soil nitrogen and applied soil nitrogen. The amount of nitrogen (N) to apply in each block was calculated using equation 3.

Residual soil N at 0 - 20 cm depth of block  $1 = \frac{\text{sum of residual N of four crop plots within block1}}{\sqrt{2}}$ 

eqn (1)

Average residual soil nitrogen in each block = residual soil N in whole study site eqn (2) N application in  $block_1 = N$  treatment of  $block_1 - residual$  soil N at 0 - 20 cm depth in  $block_1$ 

eqn (3)

Nitrogen was applied before emergence with a tractor mounted sprayer and by using liquid nitrogen fertilizer UAN (32-0-0) in all sites.

## **2.2.4.** Data collection

2.2.4.1. Stands counts: In each plot, the number of plants germinated in a meter row were counted. Plants in three one-meter rows were counted in each plot and the average number of plants m<sup>-1</sup> was calculated. Three rows in each plot were selected in such a way that they made a diagonal across the plots. Sampled area was calculated using equation 4 and plant population per square meter was calculated using equation 5.

Sampled area 
$$(m^2) = 1 (m) * row width (m)$$
 eqn (4)

Population per 
$$m^2 = \frac{average number of plants in sampled area}{sampled area (m^2)}$$
 eqn (5)

2.2.4.2. *NDVI*: Normalized Difference Vegetation Index (NDVI) is the measurement of reflectivity of plants given by;

$$NDVI = \frac{NIR - VIS}{NIR + VIS}$$
 (Bagherzadeh et al., 2020) eqn (6)

Where NIR= near-infrared, VIS= red reflectivity.

NDVI value ranges from -1 to +1 (Bagherzadeh et al., 2020). Healthy vegetation reflects more NIR but absorbs more red and blue light through chlorophyll making the NDVI value higher for

green and healthy plants (GISGeography, 2021). When there is less green vegetation and on plant maturity, the amount of chlorophyll and NDVI decreases (GISGeography, 2021).

NDVI readings were taken weekly at SAREC by using RapidSCAN CS-45 Holland scientific handheld crop sensor (Holland Scientific Inc, Lincoln NE). The NDVI unit was held one meter above the plant canopy and data was recorded by moving across the same row throughout the growing period.

2.2.4.3. *Plant stage:* The Feekes growth scale (Table 2.6; Wise et al., 2011) was used to evaluate the growth stage of all the ancient wheats and the control wheat weekly at SAREC. Only heading date was recorded at PREC and ShREC.

Table 2.6. Feekes	Growth Scale
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SARLE III sp	1111g 2017 and 2020.	
Feekes	Common Stage	Characteristics
Scale	Name	
1	Emergence	Most of the seedlings are emerged.
2-3	Tillering	Plant develops tillers. Tillers are auxiliary or side shoots
4	Green up	Plant starts to have erect growth.
5	Green up	Plant leaf sheath lengthens.
6	Jointing	Plant develops the first node at the base of the shoot.
7	Two nodes	Two nodes of the plant are visible above the soil line.
8	Flag leaf	Flag leaf appears.
9	Flag leaf ligule	Flag leaf ligule becomes visible.
10	Boot stage	Wheat head is visible inside the swollen leaf sheath.
10.5	Heading	The complete head comes out of the boot
10.5.1	Flowering	Flowering begins
10.5.3	Pollination	Pollination is complete
10.5.4	Watery ripe	Watery ripe of kernels
11.1	Milky ripe	Milky ripe of kernels
11.2	Soft dough	Kernels have doughy or mealy consistency
11.3	Hard dough	Kernels are hardened
11.4	Harvest	Ready to harvest

Table 2.6. Details of Feekes growth scale (Wise et al., 2011) used to record growth stages at SAREC in spring 2019 and 2020.

2.2.4.4. Lodging: It is the bending over of the stems near ground level of grain crops, which makes them very difficult to harvest, and may decrease potential yield. Lodging was measured by using the Horsfall-Barratt disease scoring scale (Table 2.7; Francis, 2019). This scale was developed for disease rating but was modified to be used for scoring lodging.

#### Table 2.7. Horsfall-Barratt Scale

Score	Percent Lodged	
	%	
1	0	
2	0-3	
3	3-6	
4	6-12	
5	12-25	
6	25-50	
7	50-75	
8	75-87	
9	87-94	
10	94-97	
11	97-100	
12	100	

Table 2.7. Horsfall-Barratt disease scoring scale modified to score lodging at SAREC, ShREC, and PREC in spring 2019, and 2020. Percentage of infection was modified to percentage of lodging and disease rating was modified to lodging score (Francis, 2019).

2.2.4.5. *Hail damage:* Hail damage occurred on SAREC, Lingle, WY on 9<sup>th</sup> July 2019 (Appendix 1). The number of head damaged in a meter row were counted to evaluate the hail damage. Heads in three one-meter rows were counted in each plot and the average number of heads m<sup>-1</sup> was calculated (Klein, 2021). Three rows in each plot were selected in such a way that

they made a diagonal across the plots.

2.2.4.6. *Yield and yield parameters:* Heading height from the ground to the top of the head of three plants per plot was measured. Grain samples from the center of each plot were harvested with a small plot combine. Samples were cleaned to remove chaff and weighed. Test weight was

measured using USDA test weight apparatus which is a measure of grain density (mass/volume) based on an official bushel being 1.244 cubic feet (Whitney, 2017). Wheat grains with higher test weight are considered high quality and more valuable to the end-user as they have more extractable flour and less bran (Ransom, 2017). The sampled area in hectare was calculated and yield in kg ha<sup>-1</sup> was calculated by:

$$Yield (kg ha^{-1}) = \frac{Yield (kg)}{Area (ha)}$$
eqn (7)

Hulled grain samples were dehulled using a Kimseed thresher (Kimseed Australia, Wangara WA) and cleaned by using laboratory thresher Haldrup LT-35 (Haldrup USA, Ossian IN) and soil sieves. Grain weight and grain test weight were measured, and grain yield (kg ha<sup>-1</sup>) was calculated. Percent yield loss to the hull was calculated using equation 8. Nitrogen use efficiency (NUE) is the fraction of applied nitrogen that is absorbed and used by the plant (UCDAVIS, 2021). It was calculated using equation 9.

Yield loss to hull (%) = 
$$\left[1 - \frac{\text{grain yield}}{\text{hulled yield}}\right] \times 100\%$$
 eqn (8)

 $NUE = \frac{Grain \ yield}{Residual \ soil \ nitrogen + applied \ nitrogen}$ (Moll, Kamprath, & Jackson, 1982; Appendix 3)

eqn (9)

2.2.4.7. Grain quality: For each plot in each site, 50 g full size naked grains were obtained by removing dirt, hulls, and broken grains. Then grains of each treatment from three replications were combined to make a composite 150 g sample for each treatment (nitrogen x crop x location) and sent to the California Wheat Commission lab (California Wheat Commission, Woodland CA) for protein analysis by combustion method. Grain protein was compared for each treatment. Total grain nitrogen yield was calculated by using the yield and protein content as below;

45

Grain nitrogen yield =  $\frac{Protein}{5.7}$  (Gauer et al., 1992) eqn (10)

Total grain nitrogen yield  $(kg ha^{-1}) = grain nitrogen yield \times yield (kg ha^{-1})$  eqn (11)

## 2.2.5. Data analysis

Linear mixed effect models were run separately for each species using lme4 package (Bates et al., 2015) in the R statistical language (v 3.5.1) (RStudio Team, 2020) with location, nitrogen, and the interaction between location and nitrogen as fixed factors, and year as a random factor on crop growth and yield parameters. The model is illustrated in the eqn (12) where  $Y_{ij}$  is the response for i<sup>th</sup> parameter and j<sup>th</sup> crop, location is the site where study was conducted, nitrogen is the starting soil nitrogen (eqn 3), nitrogen\*location is the interaction between nitrogen and location. For NDVI, location was removed as there was data for only one location.

$$Y_{ij} \sim nitrogen + location + nitrogen*location + |year|$$
 eqn (12)

To quantify the effect of crop species on crop growth and yield parameters a mixed linear model was run for each location separately where crop is the fixed factor and year is random factor in the model. The model is illustrated in eqn (13) where  $Y_{ij}$  is the response for i<sup>th</sup> parameter and j<sup>th</sup> location, crop is the wheat species.

$$Y_{ij}$$
~ $crop + |year|$  eqn (13)

Type III Analysis of Variance were extracted for all models. Where appropriate, logtransformation was applied to the response variable to achieve homogeneity of variances. Tukeyadjusted pairwise treatment comparisons were performed using the "emmeans" package at 5% significance level (Searle et al., 1980).

## 2.3. Results

#### 2.3.1. Crop growth

#### 2.3.1.1. Effect of nitrogen and location on crop growth

The effect of nitrogen treatment and growing location on plant stand, lodging, heading height, and days to heading was tested for each crop (Table 2.8). For all crops nitrogen and the nitrogen by location interaction had no effect on plant stands, heading height, or days to heading. Location significantly affected plant stand and lodging for einkorn, emmer, and spelt, heading height for einkorn and emmer, and days to heading for einkorn, emmer, spelt, and wheat (Table 2.8). Plant stands, and number of days to heading were consistently highest at PREC, followed by SAREC, then ShREC for all crops (Table 2.9).

Lodging of einkorn and emmer was higher at SAREC and PREC than at ShREC (Table 2.9). Lodging of spelt and wheat was the lowest at SAREC and the highest at PREC and ShREC respectively. Heading height of emmer and spelt was higher at PREC than ShREC whereas heading height of einkorn was higher at ShREC than at PREC.

#### 2.3.1.2. Effect of wheat species on crop growth

Within each location, wheat species had a significant effect on plant stand, lodging, heading height, and days to heading (Table 2.10). Plant stands of einkorn were the highest, followed by wheat/barley, emmer, then spelt, respectively (Table 2.11). Einkorn had the longest time to heading followed by spelt, emmer, and then wheat/barley. Wheat/barley and spelt had a lower lodging than other crops at SAREC and PREC and did not lodge in SAREC. However, spelt and wheat/barley had some lodging issues at ShREC and PREC. At ShREC einkorn had the lowest lodging but there was not much difference in lodging score of all species. Spelt had the highest heading height and wheat/barley had the lowest heading height in both locations.

## Table 2.8. Effects of Nitrogen and Location on Irrigated Crop Growth

Table 2.8. Analysis of variance showing P-Values for the fixed effect of nitrogen, location, and interaction between nitrogen and location on plant stands, lodging score (Table 2.7), heading height, and heading days of different spring wheat species grown during 2019 and 2020 at the three University of Wyoming research stations, SAREC, Lingle, WY, ShREC, Sheridan, WY, and PREC, Powell, WY. Analysis was done separately for each crop.

Crop	Factor	Plant	Lodging	Heading	Heading
_		Stands	Score	Height	Days
		Plant m <sup>-2</sup>	1-12	-cm-	
Einkorn	Location	0.011	0.008	< 0.001	< 0.001
	Nitrogen	0.61	0.85	0.13	1
	Location:nitrogen	0.57	0.92	0.60	1
Emmer	Location	0.017	< 0.001	< 0.001	< 0.001
	Nitrogen	0.98	0.39	0.61	0.99
	Location:nitrogen	0.28	0.97	0.36	0.99
Spelt	Location	0.003	< 0.001	0.13	< 0.001
	Nitrogen	0.58	0.84	0.26	1
	Location:nitrogen	0.45	0.93	0.47	1
Wheat	Location	0.25	0.3	$NA^1$	< 0.001
	Nitrogen	0.20	0.24	0.39	0.99
	Location:nitrogen	0.89	1	NA	1
Barley	Location	NA	NA	NA	NA
	Nitrogen	0.26	0.59	0.17	NA
	Location:nitrogen	NA	NA	NA	NA

P-Value <0.05 are significant at 5% significance level. <sup>1</sup>NA means not applicable.

## Table 2.9. Irrigated Crop Growth Comparison by Location

ShREC, Sheridan, WY and PREC, Powell, WY.							
Crop	Location	Plant Stands	Lodging	Heading	Heading		
			Score	Height	Days		
		Plant m <sup>-2</sup>	1-12	-cm-			
Einkorn	PREC	342 A	2.5 AB	89.5 B	92.9 A		
	SAREC	264 AB	3.6 A	$ND^1$	87.5 A		
	ShREC	199 B	1.2 B	108.8 A	70.0 B		
Emmer	PREC	246 A	5.6 A	106.6 A	88.6 A		
	SAREC	181 A	5.6 A	ND	70.9 B		
	ShREC	161 A	1.7 B	95.9 B	57.8 C		
Spelt	PREC	238 A	2.2 A	$NS^2$	87.9 A		
	SAREC	161 AB	1.0 B	ND	77.5 B		
	ShREC	108 B	1.2 B	NS	64.0 C		
Wheat	SAREC	NS	NS	NS	61.0 A		
	ShREC	NS	NS	NS	55.5 B		

Table 2.9. Pairwise comparison for the effect of location on plant stands, lodging score (Table 2.7), heading height, and heading days of different spring wheat species grown in 2019 and 2020 at three University of Wyoming research stations; SAREC, Lingle, WY, ShREC, Sheridan, WY and PREC, Powell, WY.

Within columns, means followed by the same uppercase letters are not different at  $\alpha$ =0.05. <sup>1</sup>ND = no data, <sup>2</sup>NS = not significant.

## Table 2.10. Effect of Wheat Species on Irrigated Crop Growth

Table 2.10. Analysis of variance showing P-Values for the fixed effect of wheat species on plant stands, lodging score (Table 2.7), heading height, and heading days at three University of Wyoming research stations, SAREC, Lingle, WY, ShREC, Sheridan, WY and PREC, Powell, WY in spring 2019 and 2020.

Location	Factor	Plant Stands	Lodging Score	Heading Height	Heading Days
		Plant m <sup>-2</sup>	1-12	-cm-	
SAREC	Crop	< 0.001	< 0.001	$ND^1$	< 0.001
ShREC	Crop	< 0.001	< 0.001	0.007	< 0.001
PREC	Crop	0.0103	0.007	< 0.001	< 0.001

P-Value <0.05 are significant at 5% significance level. <sup>1</sup>ND means no data.

## Table 2.11. Irrigated Crop Growth Comparison by Wheat Species

Table 2.11. Pairwise comparison for the effect of wheat species on plant stands, lodging score (Table 2.7), heading height and heading days at three University of Wyoming research stations, SAREC, Lingle, WY, ShREC, Sheridan, WY, and PREC, Powell, WY in spring 2019 and 2020.

Location	Crop	Plant Stands	Lodging Score	Heading Height	Heading Days
		Plant m <sup>-2</sup>	1-12	-cm-	2
SAREC	Einkorn	243 A	3.7 A	ND	87.5 A
	Emmer	188 BC	5.3 A	ND	71.0 C
	Spelt	158 C	1.0 B	ND	77.5 B
	Wheat	208 B	1.0 B	ND	61.0 D
ShREC	Einkorn	183 A	1.1 B	108.8 A	70.0 A
	Emmer	157 A	1.8 A	96.0 B	57.9 C
	Spelt	91 B	1.3 B	109.0 A	64.0 B
	Wheat	189 A	1.6 AB	77.8 C	55.4 D
PREC	Einkorn	341 A	2.6 B	96.3 B	97.0 A
	Emmer	242 B	5.6 A	105.8 A	90.0 B
	Spelt	244 B	2.2 B	109.4 A	90.0 B
	Barley	267 AB	2.1 B	54.8 C	69.0 C

Within columns, means followed by the same uppercase letters are not different at  $\alpha$ =0.05. ND means no data.

## 2.3.2. NDVI

## 2.3.2.1. Effect of nitrogen on NDVI at SAREC

Nitrogen did not affect NDVI at any major growth stages of ancient wheat and modern wheat except spelt at tillering (Table 2.12). At tillering spelt had the highest NDVI at low nitrogen treatment (0.29) followed by high nitrogen treatment (0.28) and the least at medium nitrogen treatment (0.26) at tillering stage.

## 2.3.2.2. Effect of wheat species on NDVI at SAREC:

NDVI was affected by wheat species at tillering, jointing and flag leaf stages (Table 2.13). NDVI

was the highest for einkorn at tillering and spelt at jointing and flag leaf stage (Table 2.14).

NDVI readings after the tillering stage differed between crops, indicating difference in

vegetative growth between them (Table 2.14). NDVI of einkorn did not increase much on

progressing from tillering to jointing and to flag leaf compared to other crops (Table 2.14).

# Table 2.12. Effect of Nitrogen on Irrigated NDVI

Table 2.12. Analysis of variance showing P-Values for the fixed effect of nitrogen on NDVI of different spring wheat species grown during 2019 and 2020 at the University of Wyoming James C. Hageman Sustainable Agriculture Research and Extension Center, Lingle, Wyoming. The mentioned P-Value at heading stage is for 2020 spring. There was hail damage at heading stage in spring 2019.

Crop	Factor	Tillering	Jointing	Flag leaf	Heading
Einkorn	Nitrogen	0.97	0.58	0.94	0.96
Emmer	Nitrogen	0.49	0.55	0.81	0.77
Spelt	Nitrogen	0.02	0.54	0.53	0.78
Wheat	Nitrogen	0.45	0.23	0.97	0.77

P-Value <0.05 are significant at 5% significance level.

## Table 2.13. Effect of Wheat Species on Irrigated NDVI

Table 2.13. Analysis of variance showing P-Values for the effect of crop species on NDVI at the University of Wyoming James C. Hageman Sustainable Agriculture Research and Extension Center, Lingle, WY in spring 2019 and 2020. The mentioned P-Value at heading stage is for 2020 spring. There was hail damage at heading stage in spring 2019.

Factor	Tillering	Jointing	Flag leaf	Heading	
Crop	< 0.001	< 0.001	< 0.001	0.07	

P-Value <0.05 are significant at 5% significance level.

## Table 2.14. Irrigated NDVI Comparison by Wheat Species

Table 2.14. Pairwise comparison for the effect of crop species on NDVI at the University of Wyoming James C. Hageman Sustainable Agriculture Research and Extension Center, Lingle, WY in spring 2019 and 2020.

Crop	Tillering	Jointing	Flag leaf
Einkorn	0.37 A	0.42 C	0.44 B
Emmer	0.26 B	0.53 B	0.62 A
Spelt	0.28 B	0.59 A	0.67 A
Wheat	0.28 B	0.26 D	0.50 B

Within columns, means followed by the same uppercase letters are not different at  $\alpha$ =0.05.

## 2.3.3. Yield and quality parameters

ShREC field was lost in both years due to bird damage. Crops grown at SAREC and ShREC were evaluated for the effect of nitrogen, location, and wheat species on yield and grain quality parameters.

## 2.3.3.1. Response of yield and quality parameters to nitrogen, and location:

The effect of nitrogen treatment and growing location on hulled yield, grain yield, hull loss, hulled test weight, naked grain test weight, grain protein %, and total grain N yield (kg ha<sup>-1</sup>) was tested for each crop (Table 2.15). For all crops nitrogen and the nitrogen by location interaction had no effect on yield and quality parameters except the hulled test weight of einkorn (Table 2.15). The hulled test weight of einkorn at PREC in 56 kg ha<sup>-1</sup> nitrogen treatment (14.3 kg) was significantly higher than hulled test weight of einkorn at SAREC in 110 kg ha<sup>-1</sup> nitrogen (12.7) kg), 90 kg ha<sup>-1</sup> nitrogen (12.7 kg), and 56 kg ha<sup>-1</sup> nitrogen treatment (12 kg). There was no significant difference between hulled test weights of einkorn at PREC at 28 and 90 kg ha<sup>-1</sup> nitrogen treatments, and SAREC at 110 and 90 kg ha<sup>-1</sup> nitrogen treatments (14.1 kg). Location significantly affected all yield and quality parameters except hull loss and grain test weight of einkorn, and total grain nitrogen yield of emmer and spelt (Table 2.15). Hulled yield, grain yield, and hulled test weight were consistently highest at PREC than at SAREC (Table 2.16). Grain protein % and yield loss to hull was higher at SAREC than PREC for all crops (Table 2.16). Grain test weight of emmer and spelt and total grain N yield of einkorn was higher at PREC than at SAREC (Table 2.16). However, total grain N yield of emmer and spelt was similar in both locations (Table 2.16).

## Table 2.15. Effect of Nitrogen and Location on Irrigated Yield and Quality

Table 2.15. Analysis of variance showing P-Values for the fixed effect of location, nitrogen, and interaction between location and nitrogen on hulled yield, grain yield, loss, hulled test weight, grain test weight, grain protein %, and total grain N yield of different spring wheat species in 2019 and 2020 at two University of Wyoming research stations, SAREC, Lingle, WY and PREC, Powell, WY. Plots at ShREC, Sheridan, WY were lost in both years due to bird damage.

Parameter	Factor	Einkorn	Emmer	Spelt	Wheat	Barley
Hulled			Kg ha <sup>-1</sup>			
Yield	Location	< 0.001	< 0.001	0.03	$\mathbf{N}\mathbf{A}^{1}$	NA
	Nitrogen	0.46	0.57	0.84	NA	NA
	Location:nitrogen	0.14	0.39	0.09	NA	NA
Grain Yield				Kg ha <sup>-</sup>	1	
	Location	< 0.001	< 0.001	< 0.001	NA	NA
	Nitrogen	0.72	0.61	0.75	0.62	0.06
	location:nitrogen	0.69	0.42	0.13	NA	NA
Loss			%			
	Location	0.14	< 0.001	< 0.001	NA	NA
	Nitrogen	0.5	0.63	0.80	NA	NA
	location:nitrogen	0.07	0.34	0.94	NA	NA
Hulled Test			Kg/busł	nel		
Weight	Location	< 0.001	0.012	< 0.001	NA	NA
	Nitrogen	0.88	0.92	0.91	NA	NA
	location:nitrogen	0.04	0.60	0.89	NA	NA
Grain Test				Kg/bushel		
Weight	Location	0.43	0.002	< 0.001	NA	NA
	Nitrogen	0.45	0.73	0.82	0.67	0.054
	location:nitrogen	0.97	0.38	0.92	NA	NA
Protein				%		
	Location	< 0.001	< 0.001	< 0.001	NA	$ND^2$
	Nitrogen	0.7	0.43	0.96	0.5	ND
	location:nitrogen	0.5	0.35	0.90	NA	ND
Total Grain	nKg ha <sup>-1</sup>					
Nitrogen	Location	0.04	0.83	0.46	NA	ND
Yield	Nitrogen	0.52	0.2	0.28	0.83	ND
	location:nitrogen	0.85	0.59	0.23	NA	ND

P-Value <0.05 are significant at 5% significance level. <sup>1</sup>NA means not applicable. <sup>2</sup>ND means no data.

#### Table 2.16. Irrigated Yield and Quality Comparison by Location

Table 2.16. Pairwise comparison for the fixed effect of location on hulled yield, grain yield, loss, hulled test weight, grain test weight, grain protein %, and total grain N yield at two University of Wyoming research stations, PREC, and SAREC in spring 2019 and 2020.

Parameter	Location	Einkorn	Emmer	Spelt	
Hulled Yield			Kg ha <sup>-1</sup>		
	PREC	3325.0 A	3397.0 A	2610.0 A	
	SAREC	1063.0 B	2007.0 B	2004.0 B	
Grain Yield		Kg ha <sup>-1</sup>			
	PREC	2401A	2660.0 A	1882.0 A	
	SAREC	357 B	1369.0 B	1066.0 B	
Loss			%%		
	PREC	35.9 A	20.9 B	27.8 B	
	SAREC	39.2 A	31.3 A	48.7 A	
Hulled Test		Kg/bushel			
Weight	PREC	14.1 A	16.9 A	14.6 A	
	SAREC	12.4 B	15.3 B	10.9 B	
Grain Test			Kg/bushel		
Weight	PREC	26.9 A	27.2 A	28.2 A	
	SAREC	27.4 A	26.5 B	27.2 B	
Protein		%%			
	PREC	10.5 B	9.8 B	12.1 B	
	SAREC	17.9 A	17.0 A	18.2 A	
Total Grain N		Kg ha <sup>-1</sup>			
Yield	PREC	44.5 A	44.8 A	38.5 A	
	SAREC	13.5 B	43.9 A	36.7 A	

Within columns, means followed by the same uppercase letters are not different at  $\alpha$ =0.05.

#### 2.3.3.2. Response of yield and quality parameters to wheat species:

Within each location, wheat species had significant effect on hulled yield, grain yield, loss, hulled test weight, grain test weight, grain protein %, total grain N yield (kg ha<sup>-1</sup>) except hulled yield and total grain N yield (kg ha<sup>-1</sup>) at PREC and grain test weight at SAREC (Table 2.17). All the ancient wheats had similar grain yield and similar grain N at PREC (Table 2.18). At SAREC, einkorn had consistently lower grain yield and grain N than other species. At PREC, einkorn and

emmer had higher hulled yield than spelt (Table 2.18). Yield loss to hull was highest for einkorn followed by spelt and then emmer at PREC whereas it was highest for spelt, followed by einkorn and then emmer at SAREC (Table 2.18). Hulled test weight was higher for emmer than einkorn and spelt in both locations. All the wheat species had similar grain test weight at SAREC whereas spelt had the highest grain test weight followed by emmer, einkorn and then barley at PREC (Table 2.18). All the wheat species except emmer had similar protein % in both locations. Spelt had the highest protein content in both locations which was statistically similar to einkorn, and modern wheat and higher than emmer at SAREC (Table 2.18).

## Table 2.17. Effect of Wheat Species on Irrigated Yield, and Quality

Table 2.17. Analysis of variance showing P-Values for the fixed effect of location, nitrogen, and interaction between location and nitrogen on hulled yield, grain yield, loss, hulled test weight, grain test weight, grain protein, and total grain N yield of different spring wheat species in 2019 and 2020 at two University of Wyoming research stations, SAREC, Lingle, WY and PREC, Powell, WY.

Parameter	Factor	PREC	SAREC	
Hulled Yield		]	Kg ha <sup>-1</sup>	
	Crop	0.011	0.0014	
Grain Yield		]	Kg ha <sup>-1</sup>	
	Crop	< 0.001	< 0.001	
Loss			-%	
	Crop	< 0.001	< 0.001	
Hulled Test Weight		]	Kg/bushel	
	Crop	< 0.001	< 0.001	
Grain Test Weight			Kg/bushel	
	Crop	< 0.001	0.74	
Protein		%%		
	Crop	< 0.001	< 0.001	
Total Grain N Yield		Kg ha <sup>-1</sup>		
	Crop	0.37	< 0.001	

P-Value <0.05 are significant at 5% significance level.

# Table 2.18. Irrigated Yield and Quality Comparison by Location

Table 2.18. Pairwise comparison for the fixed effect of wheat species on hulled yield, grain yield, loss, hulled test weight, grain test weight, grain protein, and total grain N yield at two University of Wyoming research stations, PREC, Powell, WY and SAREC, Lingle, WY in spring 2019 and 2020.

	Crop	PREC	SAREC
Hulled Yield		Kg ha <sup>-1</sup>	
	Einkorn	3277.0 A	1139.0 B
	Emmer	3274.0 A	2123.0 A
	Spelt	2585.0 B	2078.0 A
	Control	NA	NA
Grain Yield		ŀ	Kg ha <sup>-1</sup>
	Einkorn	2337.0 B	450.0 B
	Emmer	2596.0 B	1448.0 A
	Spelt	1844.0 B	1123.0 A
	Control	4740.0 A	1295.0 A
Loss			-%
	Einkorn	35.9 A	39.2 B
	Emmer	21.0 C	31.4 C
	Spelt	28.7 B	48.3 A
	Control	NA	NA
Hulled Test Weight		Kg/	/bushel
C	Einkorn	13.9 B	12.5 B
	Emmer	17.0 A	15.2 A
	Spelt	14.5 B	11.0 C
	Control	NA	NA
Grain Test Weight		Kg/	/bushel
C	Einkorn	27.0 B	27.4 A
	Emmer	27.1 B	26.6 A
	Spelt	28.1 A	27.2 A
	Control	23.4 C	27.2 A
Protein			_%
	Einkorn	10.6 B	18.0 A
	Emmer	9.8 B	17.1 B
	Spelt	12.2 A	18.3 A
	Control	NA	18 A
Total Grain N Yield		Kg ha <sup>-1</sup>	
	Einkorn	43.0 A	14.5 B
	Emmer	44.8 A	43.9 A
	Spelt	38.5 A	36.7 A
	Control	ND	40.8 A

Within columns, means followed by the same uppercase letters are not different at  $\alpha$ =0.05.

#### 2.4. Discussion

In the past decades the ancient wheats, einkorn, emmer, and spelt have received increased attention from consumers, bakers, breeders, and farmers because of their potential health benefits and sustainable production. A successful re-establishment of einkorn, emmer, and spelt production requires knowledge about best farming practices and quality parameters that are important for bakery and pasta production. Wyoming farmers need to understand the nitrogen demand, agronomic characteristics, and quality performance of ancient wheats under multiple Wyoming growing conditions and locations. The ancient wheats are old species and less domesticated than modern wheat and barley. It is thus not surprising to see differences in heading time, growth and yield parameters of these species compared to modern wheat and each other.

### 2.4.1. Nitrogen treatment had no effect on growth, yield and quality

We found no effect of nitrogen on any growth, yield, and quality parameters (Table 2.8 and 2.15). Previous experiments (Castagna et al., 1995, 1996), showed a similar response where the yield of ancient and modern wheat did not increase with increasing nitrogen application from 0-120 kg ha<sup>-1</sup>. Alemu & Bayisa (2016) reported that emmer had the highest yield at the low nitrogen rate (0-23 kg ha<sup>-1</sup>) compared to 46-69 kg ha<sup>-1</sup>. They also found no significant difference among the four levels of nitrogen application and their impact on tillers m<sup>-2</sup>, spike length, number of spikes m<sup>-2</sup>, seeds spike<sup>-1</sup>, plant height, and days to maturity. Pearman et al. (1977) similarly reported that grain yield of wheat was not affected by addition of nitrogen from 0-200 kg N ha<sup>-1</sup> in two years of their study. Tanaka & Nakano (2019) found that barley yield was increased with increasing nitrogen in one cropping season but not in another season. Their findings are similar to the results we report here. Alemu & Bayisa (2016) suggested that increasing the level of nitrogen fertilizer leads to increase in height of plants, production of weak

and succulent plants, increase susceptibility to lodging and diseases all of which results further decrease in grain yield and quality. However, plant height and lodging were not affected by nitrogen in our study. Several factors, or combination of several factors, could have resulted nitrogen loss in our study. Nitrogen application was done pre-planting by sprayer without incorporation using liquid urea ammonium nitrate (UAN). Any surface applied ammonia- and ammonium-based nitrogen fertilizer can lose nitrogen to the atmosphere via ammonia volatilization and the potential is greatest with urea and fluids containing urea such as UAN (Jones, Brown, Engel, Horneck, & Olson-Rutz, 2020). We used urease inhibitor to control ammonium loss, but it restricts urease hydrolysis only up to 7-14 days after which rain, irrigation, or soil mixing would be required to further restrict ammonia losses (IPNI, 2021). Soil temperature was very low (5-9°C in Lingle in 2019 and 2020, 2°C in Powell in 2020) (WACNet, 2021) when we applied nitrogen which has been shown to effect plant response to nitrogen. We do not have soil temperature for Powell in 2019. But we assume the soil temperature in 2019 and 2020 were similar as the air temperature was similar (Figure 2.1). Jones, Brown, Engel, Horneck, & Olson-Rutz, (2020) reported that peak nitrogen loss occurred in their study when soil temperature was below 5°C. There is low microbial activity at low soil temperature which decreases ammonization and nitrification process, decreasing the amount of available nitrogen to plants (Havlin, Tisdale, Nelson, & Beaton, 2013). Optimum soil temperature for microbial activity is 20-40°C (Havlin, Tisdale, Nelson, & Beaton, 2013). Besides, soil stays moist longer at low temperature and ammonia which stays in solution volatilize slowly in such condition (Jones, Brown, Engel, Horneck, & Olson-Rutz, 2020). Furthermore, both of our study sites had alkaline pH (Table 2.2) and higher pH can decrease the nitrification process (Havlin, Tisdale, Nelson, & Beaton, 2013). The pH of soil can also change

over the growing season by application of fertilizers and rainfall affecting the processes by which nitrogen is available to crops (Havlin, Tisdale, Nelson, & Beaton, 2013). Incorporating nitrogen source in soil, late planting to avoid low soil temperature and decreasing soil pH by adding elemental sulfur, aluminum sulfate or sulfuric acid can help to reduce nitrogen loss and obtain benefit of nitrogen addition. Nitrogen application timing has also been shown to be critical for effectiveness (Lopez-Bellido, Fuentes, Castillo, Lopez-Garrido, & Fernandez, 1996). Split application of nitrogen may reduce nitrogen losses and lead to a better translocation of preanthesis assimilates to the grain (Abdin et al., 1996). Castagna et al. (1996) applied a split nitrogen application and found that the protein content increased on increasing nitrogen but the grain yield remained constant. Marino et al. (2011) reported that nitrogen application at tillering increased net grain yield of emmer by 36% in comparison to nitrogen application at seeding and stem elongation stages. Emmer also performed the best when a 3-stage split application of nitrogen fertilizer (30-30-30 kg ha<sup>-1</sup>) was performed at seeding, tillering, and stem elongation stages in their study.

Protein content in our study was 14-18% for all the wheat species, even at the low nitrogen treatment, which is similar to average protein percent reported in other studies. Longin et al. (2016) reported a similar finding about ancient wheats in which einkorn, emmer, and spelt had higher protein content than bread wheat, even though they received a nitrogen fertilizer amount reduced by 75, 75, and 35%, respectively, compared to bread wheat. The ancient wheats and modern wheat were able to maintain protein quality even at low nitrogen application in our study demonstrating that they have potential to produce high grain protein even at low nitrogen application to nitrogen treatments which suggests either low nitrogen was sufficient in those sites or applied

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nitrogen was lost to the environment or not available to the plants. Measures to minimize nitrogen loss such as incorporation of nitrogen source at a lower depth, adding sulfur products to decrease pH, and split nitrogen application should be adapted (Combs, 2021). Future studies using different nitrogen rates and adapting measures to reduce nitrogen loss can be done to better elucidate the nitrogen demand of these ancient wheats.

## 2.4.2. Location affected the growth, yield, and quality

Location had significant effect on growth, yield, and quality parameters of ancient and modern wheat (Table 2.8 and 2.15). Hlisnikovský et al. (2019) and Castagna et al. (1996) reported that climate and soil characteristics had effect on yield parameters and grain composition in their study, supporting that ancient wheat will perform differently across multiple growing locations. For all the crops, the heading period was longer at PREC than at SAREC (Table 2.9). The accumulation of average daily temperatures is calculated as 'growing degree days (GDD)' and wheat should accumulate certain growing degree days to reach certain stage (NDAWN, 2021). PREC reached the same GDD later in the season than SAREC which resulted in a longer heading period at PREC than at SAREC (Table 2.9 and 2.19). Grain yield of einkorn, emmer, and spelt were 6.7, 1.94, 1.76 times higher at PREC than at SAREC, respectively (Table 2.16). Several factors could be responsible for the yield differences in two locations. Growing degree day for all crops was higher at SAREC than at PREC after heading (grain filling period) (Table 2.19). The average temperature at SAREC from heading to 15 days after heading ranged from 19-25 °C in 2019 and 19-27 °C in 2020 (WACNet, 2021). The temperature at the same growth period at PREC ranged from 14-24 °C in 2019 and 11.2-24 °C in 2020 (NOAA, 2021). For every 1°C increase above a mean temperature of 23°C wheat yield can decrease by around 10% (Gibson & Paulsen, 1999). The temperature above 23 lasted for 5-10 days for all crops in Lingle and 1-3

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days in Powell in both years. Prasad & Djanaguiraman, (2014) reported that plants exposed to temperatures above 24°C for the period of 5 days at the start of heading significantly reduced floret fertility and there was significant decrease in floret fertility and individual grain weight with increased duration of high temperature stress from 0-30 days from heading. Higher daily temperature at SAREC than PREC for longer period can be one of the reasons for lower yield at SAREC than at PREC. Higher plant stands at PREC than at SAREC (Table 2.9) could also be another reason for yield differences. In cereals, higher plant stands result in fewer tillers, which may increase uniformity in flowering and maturity and increase yield and quality (Lovell, 2020). Besides Powell was irrigated with flood irrigation whereas SAREC was irrigated with sprinkle irrigation which may also have affected yield. Soil types could also have affected performance in two locations. SAREC had Haverson and McCook loams soil (loam from 0-30 cm and stratified sandy loam 30-152 cm) (Soil survey staff, 2021). Powell had garland loam (loamy soil from 0-23 cm +clay loam 23-43 cm + loam 43-74 cm) (Soil survey staff, 2021). Loamy soil and clay loam are the most suitable soil types to grow wheat (Britannia Inc., 2021). Sandy loam has a higher portion of sand which is unable to hold water and susceptible to leaching loss of many nutrients and additional fertilizers (Joel, 2021). Difference in soil type might be another reason for lower yield at SAREC than at PREC. SAREC also had lower magnesium content in soil (Table 2.2). Magnesium deficiency reduces the leaf growth rate, affecting the assimilate supply to growing roots and their capacity to acquire nutrients and ultimately decreases the yield (Cakmak et al., 1994). Lower magnesium at SAREC might be another reason for their lower yield compared to PREC though we did not see any deficiency symptoms in our plots (Table 2.2). Any of the factors above or combination of them could be the reason for higher yield at PREC than at SAREC.
These findings support that Powell, WY is a more suitable location to grow spring einkorn, emmer, and spelt than Lingle, WY. Previously, researchers found that yield of spring wheat was higher in Powell than in Lingle, WY supporting our finding that Powell, WY is more suitable location to grow both ancient and modern spring wheat than Lingle, WY. The average grain yield of spring wheat produced in Powell WY in 2009 and 2010 was 7808 kg ha<sup>-1</sup> and 7498 kg ha<sup>-1</sup> respectively (Killen & Violett, 2010; Killen, 2009a) whereas the average grain yield of spring wheat produced in Lingle, WY was 3161 kg ha<sup>-1</sup> (Killen, 2009b). The yield of barley grown at PREC in our study was lower than the average yield reported at PREC in past studies. Our barley yield at PREC (4740 kg ha<sup>-1</sup>) is 1.2 times lower than average yield (6052 kg ha<sup>-1</sup>) in 2002 (Bjornestad, Killen, Hybner, & Natchman, 2002) and nearly half of the yield (9005 kg ha<sup>-</sup> <sup>1</sup>) in 2011 (Killen, Mesbah, & Violett, 2011). The average yield of modern wheat (1295 kg ha<sup>-1</sup>) at SAREC in our study was one third of the average yield (3161 kg ha<sup>-1</sup>) reported in Lingle in 2009 (Killen, 2009b). Hail damage in 2019 (Appendix 1), and non-uniform maturation of modern wheat (Figure 2.4) may be reasons for such a low yield at SAREC. Around 25% of the modern wheat heads were green when 75% of the heads were fully matured at harvest at SAREC in 2019 (Data not shown). The yield of ancient wheats at both locations in our study was also lower than the yield reported in other studies. Chapagain & Riseman (2012) reported that the grain yield of irrigated spring einkorn and emmer were 2800 kg ha<sup>-1</sup> and 3850 kg ha<sup>-1</sup> <sup>1</sup>respectively. In our study, einkorn and emmer yield were 1.2 and 1.5 times lower than their study. Similarly, spelt yield in our study was also comparatively lower. However, none of the grains meet the expected yield in our study which might be due to impact of growing year. The ancient wheats yield might go higher in other years having favorable growing conditions.

While the grain yield was higher at PREC, the percent protein was higher at SAREC for all the crops. Protein % is inversely proportional to grain yield in cereals (Blanco et al., 2012). This could be the reason for the higher grain protein % of all wheat species at SAREC than at PREC.

The total grain nitrogen yield of emmer and spelt was similar in both locations whereas total grain nitrogen yield of einkorn was higher at PREC than at SAREC (Table 2.16). The total grain nitrogen yield is the product of yield and grain nitrogen [eqn (10 and 11)]. The nitrogen uptake by ancient wheats that could not be used to increase plant yield in SAREC was accumulated in grains as grain nitrogen and that increased the protein % of the grain. For spelt and emmer, yield at PREC was 1.8 and 1.9 times higher than at SAREC, respectively, and the grain nitrogen was 1.7 and 1.5 times lower than at SAREC making the total grain nitrogen yield in both locations similar (Table 2.16). However, for einkorn yield at PREC was 6.7 times higher than at SAREC was only 1.7 times lower than at SAREC making the total grain nitrogen higher total grain nitrogen higher that states the grain nitrogen at PREC was only 1.7 times lower than at SAREC making the total grain nitrogen higher total grain nitrogen higher at PREC than at SAREC (Table 2.16).

Powell is a more suitable location to grow both modern spring grains and spring ancient wheats than Lingle, WY. Yield of ancient wheat in both locations was lower than yield reported at other locations in past studies. However, the yield of the modern grains was also lower than past studies in our study sites suggesting that the yield of both ancient and modern wheat were lower than may be expected.

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# Table 2.19. Growing degree day in irrigated sites

research stations, SAREC, Lingle, WY and PREC, Powell, WY in spring 2019 and 2020.										
Crop	GDD till		GDD 1	GDD 15 days		n heading	Whole	Whole season		
	heading		after he	after heading		rvest	GE	GDD		
	SAREC	PREC	SAREC	PREC	SAREC	SAREC PREC		PREC		
				201	9					
Einkorn	1245	1301	336	325	1037	1069	2262	2347		
Emmer	973	1128	334	277	969	852	1924	1961		
Spelt	1111	1128	335	277	913	852	2002	1961		
Control	859	1128	318	277	975	975 742		1851		
2020										
Einkorn	1505	1117	350	298	1027	1056	2506	2158		
Emmer	1019	1005	336	264	853	951	1853	1944		
Spelt	1174	1005	355	264	1356	951	2506	1944		
Control	758	684	278	209	1020	1273	1761	1944		

Table 2.19. Growing degree day (GDD) of wheat species grown at two University of Wyoming research stations, SAREC, Lingle, WY and PREC, Powell, WY in spring 2019 and 2020.

# 2.4.3. Each ancient wheat species has unique growth, yield, and quality

Crop species significantly affected growth, yield, and quality parameters in both locations (Table 2.10 and 2.17). Plant stand of einkorn was the highest and plant stand of spelt was the lowest (Table 2.9). Hull size of spelt is relatively bigger and hull size of einkorn is relatively smaller than other species (Figure 2.5) which might be the reason for difference in plant stands as plots were seeded on a weight basis. Ancient species had higher lodging than the modern wheat and barley (Table 2.11) which can be a problem in handling and growing ancient wheats. In our study, ancient wheats were 20-56 cm taller than the modern wheat/barley. Greater heights increase the susceptibility to lodging and reduce plant ability to complete grain ripening (Troccoli & Codianni, 2005). Thus greater height can be one of the reason for lodging of ancient wheats. Longin et al. (2016) had a similar finding in which ancient wheats were 30 cm taller and experienced lodging issues compared to modern wheat. However, there are semi-dwarf ancient wheats with less lodging issues (Konvalina et al., 2010). Emmer and spelt had higher NDVI than modern wheat in all the stages suggesting that they had higher vegetative growth and potential to

grow as forage crop than modern wheat (Table 2.14). Cadeddu et al. (2021) clipped the above ground biomass (herbage) from einkorn and emmer early in the season to feed animals and found no yield decline at harvest. This suggests that these ancient wheats could be used for both forage and grain dual purpose (grain+forage).

In all locations, wheat headed first, followed by emmer, spelt, and then einkorn last. Einkorn took 17-28 days more to reach heading stage than modern wheat (Table 2.11). Emmer and spelt took 2-21 days more than modern wheat to reach heading stage (Table 2.11). Chapagain & Riseman (2012), Castagna et al. (1996), and Longin et al. (2016) reported a similar finding in which ancient wheats matured one to four weeks later than modern wheat. Hence, growing ancient wheats may require alteration of some management and crop rotation practices to account for a longer growing season.

Grain yield of all ancient wheats was similar in PREC (Table 2.18). However, their yield was half of the barley yield. The yield of emmer and spelt was similar to modern wheat at SAREC. But the yield of all the wheat species was lower than expected. Protein content of the spelt was the highest among all the wheat species in both locations (Table 2.18). However, the difference was significant only at SAREC. Ancient wheats typically have higher protein content than the modern wheat. However, in our study we found that ancient wheat had similar protein content compared to modern wheat. Castagna et al. (1996) reported a similar finding in which ancient wheats were not more efficient in protein accumulation than modern wheat. Other varieties of ancient wheats than the varieties grown in our study might be more efficient in protein accumulation than the nutritional differences a more in-depth nutrient analysis needs to be done on the grains. The low yield of the wheat in our study might have resulted in higher than average (~18%) protein content among all varieties. Hence, further

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studies in other favorable growing years, and by using other varieties of ancient wheats is needed to determine the potential of each ancient wheat in irrigated fields of Wyoming.



Figure 2.4. Non-uniform Maturation of Modern Wheat

Figure 2.4. Modern wheat growing in 2020 at SAREC, Lingle, WY showing non-uniform maturation.



Figure 2.5. Planting Form of Ancient and Modern Wheats

Figure 2.5. Planting form of ancient and modern wheats. A. Modern wheat B. Spelt C. Emmer D. Einkorn. Ancient wheats are seeded in hull. Modern wheat is seeded with naked grain. Each ancient wheat has unique hull size. Spelt, and emmer have two grains hull<sup>-1</sup>. Einkorn has one grain hull<sup>-1</sup>.

#### **2.5.** Conclusion

In the present study, ancient wheats were grown together with modern wheat/barley under irrigation, in three different growing regions of Wyoming, and under different nitrogen treatments. This study allowed comparisons of three different ancient wheat performance with each other and locally grown modern grain varieties under different nitrogen treatments, and growing locations. Pre-planting surface application of nitrogen had no effect on growth, yield, and quality parameters suggesting either nitrogen was lost to environment or unavailable to plants or their yield potential can be obtained even at low nitrogen treatments used in our study. Yield for all crops was consistently higher in Powell, WY than Lingle, WY suggesting that Powell is a better suited location to grow these ancient wheats. In both locations, the yield of all crops was lower than expected and compared to other studies in different locations under irrigation. This suggests that the yield difference might be due to the growing year and the yield may increase in favorable growing years. Ancient wheats matured more slowly than modern wheat with einkorn being the slowest. Timing of agronomic management practices will need to be considered if ancient grains are introduced in crop rotation practice in WY. In our study, the ancient wheats did not have higher protein content than modern wheat. This suggests that it might not be worth growing ancient wheats with lower yield and similar protein content unless they have very high market price. Besides, the poor yield suggests repeating the study with some modifications in order to know the true potential of growing ancient wheats in Wyoming. Studies with measures to reduce nitrogen loss such as soil nitrogen incorporation, split N application, additional varieties, and more locations are necessary to understand the true potential of ancient wheat in Wyoming.

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# CHAPTER 3. ANCIENT WHEAT PRODUCTION IN DRYLAND FIELDS OF WY

#### **3.1. Introduction**

Small grains are widely grown in Wyoming dryland (USDA, 2019, 2020). In 2017, 9,051 acres of barley, 86,128 acres of winter wheat, and 6,092 acres of spring wheat were grown in dryland fields of Wyoming (USDA, 2019). However, consumer preferences are changing from the modern small grains to ancient wheat products because of their unique taste and higher nutritional benefit compared to the modern wheat (Boukid et al., 2018). Einkorn (*Triticum monococcum* L.), emmer (*Triticum turgidum* L.), and spelt (*Triticum spelta* L.) are ancient species of wheat that are currently receiving increased attention (Shewry & Hey, 2015). These wheats have historically been cultivated under low-input marginal conditions (Bencze et al., 2020) and hypothesized to perform better in low fertility and dry soil compared to modern wheat (Boukid et al., 2018; Stagnari et al., 2008; Troccoli et al., 1997).

There can be significant differences in plant growth characteristics, maturity, agronomic performance, and input requirement of ancient wheats compared to modern wheat as they have less breeding and crop development than modern wheat (Arzani & Ashraf, 2017; Longin et al., 2016). Ancient wheats are hulled and not free threshing hence requiring an extra dehulling process before milling or malting (Longin et al., 2016). They have lower yield than modern wheat (Okuno et al., 2014), however, the relative economic return associated with ancient wheats can be greater than modern wheat due to high market demand and prices (Cadeddu et al., 2021). Konvalina et al. (2014) reported that average yields of einkorn, emmer, spelt, and modern bread wheat were 1640 kg ha<sup>-1</sup>, 2430 kg ha<sup>-1</sup>, 2970 kg ha<sup>-1</sup>, and 3470 kg ha<sup>-1</sup> respectively under organic

and low input farming. Bencze et al. (2020) reported that the average yield of emmer and einkorn were similar, 2830 kg ha<sup>-1</sup> and 2820 kg ha<sup>-1</sup> respectively, but their yield varied by growing year. Longin et al. (2016) reported that the mean grain yield of fifteen accessions each of bread wheat, durum wheat, spelt, emmer, and einkorn were 8000, 6100, 5000, 3600, and 2700 kg ha<sup>-1</sup>, respectively in southern Germany. This suggests that each ancient wheat has unique performance, but the performance may vary by year, soil, and climatic condition.

Ancient wheats have more of a tendency to lodge than modern wheat. Longin et al. (2016) observed lodging issues in einkorn, emmer, and spelt, but not in modern wheat. Higher lodging of ancient wheats can result in poor performance compared to modern wheat. Konvalina et al. (2010) noticed that different varieties of ancient and modern wheat had different lodging resistance. Choosing ancient wheat varieties with less lodging issues can help to increase the yield. Ancient wheats also have slower maturation than modern wheat. Castagna et al. (1996) reported that modern bread wheat reached heading ten days earlier than emmer, sixteen days earlier than spelt, and 24 days earlier than einkorn. Troccoli & Codianni (2005) had a similar finding for ancient wheats in which emmer headed first, followed by spelt, and then einkorn. Ancient wheats have a longer growth period than modern wheat and replacing modern wheat with ancient wheat might require some alteration in the common crop rotation practices, agronomic management practices, and timing of intercultural operations.

Some research has found ancient wheats have high nitrogen use efficiency and perform better even at low N application rate (Pourazari et al., 2015). Despite their history, there are contrasting results from research on the optimal nitrogen requirements for ancient wheats. Marino et al. (2016) found that increasing nitrogen from 0-90 Kg ha<sup>-1</sup> increased yield of emmer lines. Marino et al. (2011), also reported that increasing N rates from 60-90 Kg ha<sup>-1</sup> increased hulled yield,

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grain yield, and the total protein content of emmer under rainfed conditions. However, Fatholahi et al. (2020) reported that all the ancient wheats were unresponsive to nitrogen application (0-120 Kg ha<sup>-1</sup>). Castagna et al. (1996) found that increasing nitrogen significantly increased the biomass yield, grain protein of the hulled wheats but did not increase the grain yield. However, the cultivar, location, and several other factors might have affected the nitrogen use in these studies. Due to contrasting results in the research done to date, it is clear that more and regionally specific studies are needed to understand the nitrogen requirement of ancient wheats.

Ancient wheats have been shown to have high water use efficiency and suitability for marginal and organic farming (Vaghar & Ehsanzadeh, 2018). Konvalina et al. (2012) reported that all emmer accessions used in their study had higher resistance to drought than the modern wheat. Vaghar & Ehsanzadeh (2018) reported that modern wheat had severe water stress symptoms on 30-40% and 60-70% of depletion of available soil water whereas emmer showed minimal modification on photosynthetic pigments on water stress. They also found that modern wheat had higher water use efficiency (WUE) under high nitrogen application whereas emmer showed no change in WUE on high nitrogen application vs no application in their study. The performance of emmer seemed to be affected less than modern wheat at low nitrogen and water conditions.

Ancient wheats can be an alternative crop in dryland Wyoming based on the success of other small grains. Lower yield of ancient wheats compared to modern wheat should be offset by premium markets and higher price than modern wheat. Each ancient wheat is likely to have unique performance in different growing regions. The yield potential and best management practices for dryland ancient wheat production in Wyoming is not known. Furthermore, to the best of our knowledge studies on agronomic and quality performance of hulled wheats across multiple dryland locations are lacking in the literature. The aim of our study was to identify

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agronomic management practices and nitrogen demand of spelt, emmer, and einkorn and how nitrogen affects agronomic traits of these ancient wheats under multiple Wyoming growing locations under dryland conditions. Our research questions are;

- Which ancient wheat is best suited for Wyoming dryland conditions?
- Do ancient wheats perform differently in different growing regions in WY?
- Are ancient grains able to maintain yield and quality in low N treatments under dryland conditions?

# 3.2. Materials and methods

# 3.2.1. Study site

This study was conducted in spring 2019 and 2020 at two University of Wyoming research stations, the James C. Hageman Sustainable Agriculture Research and Extension Center (SAREC) in Lingle, WY, and the Sheridan Research and Extension Center (ShREC) in Sheridan WY (Table 3.1). Fields were rainfed in both locations. The average monthly temperature and total monthly precipitation for the spring growing season (April-August) are shown in Figure 3.1 and Figure 3.2, respectively. The average monthly temperature was similar in both stations (Figure 3.1). In both locations, precipitation was higher in 2019 during early vegetative growth stage (May), and reproductive stages (July, August) than in 2020 (Figure 2). In 2019, precipitation was higher in SAREC than ShREC during reproductive stages (July and August). SAREC had textural class of silt loam at 0-3 cm and loamy soil at 3-150 cm (Soil survey staff, 2021). Soil textural class of Wyarno was the combination of loam, silt loam and clayloam (Soil survey staff, 2021). Soil properties are described in Table 3.2.

#### Table 3.1. Coordinates, Elevation, and Nitrogen Treatments in Dryland Sites

Table 3.1. Coordinates, elevation, and nitrogen treatments at two dryland study sites,											
James C. Hageman Sustainable Agriculture Research and Extension Center (SAREC),											
Lingle, WY, and Sheridan Research and Extension Center (ShREC), Sheridan, WY in											
spring 2019, and spring 2020. Coordinates and elevation were obtained from google.											
Location	Coordinates	Elevation	Nitrogen Treatments								
			High N Medium N Lov								
	mKg ha <sup>-1</sup>										
SAREC	42.07 N, 104.38 W	1,272	90	56	28						
ShREC	44.83 N,106.82 W	1,174	90	56	28						



Figure 3.1. Mean Monthly Temperature (°C) in Dryland Sites

Figure 3.1. Mean monthly temperature (°C) from planting to harvesting of the crop in spring 2019, and spring 2020 at two study sites, SAREC, Lingle, WY, and ShREC, Sheridan, WY. Data was acquired from NOAA National Center for Environmental information.



Figure 3.2. Total Monthly Precipitation (mm) in Dryland Sites

Figure 3.2. Total monthly precipitation (mm) from planting to harvesting of the crop in spring 2019, and spring 2020 at two study sites; SAREC, Lingle, WY, and ShREC, Sheridan, WY. Data was acquired from NOAA National Center for Environmental information

# Table 3.2. Dryland soil properties

Sail properties	SA	REC	ShREC		
Son properties	2019	2020	2019	2020	
pH	8.3	8.2	7.5	7.6	
Organic matter (%)	2.3	2.2	2.7	2.2	
Phosphorus (mg kg <sup>-1</sup> )	52	57	118	77	
Potassium (kg ha <sup>-1</sup> )	757	674	372	213	
Calcium (mg kg <sup>-1</sup> )	4213	4070	3037	3263	
Magnesium (mg kg <sup>-1</sup> )	203	200	1038	362	
Cation exchange capacity [meq (100g) <sup>-1</sup> ]	24.7	23.7	24.8	19.9	

Table 3.2. Chemical properties of soil at two study sites, SAREC, Lingle, WY, and ShREC, Sheridan, WY in spring 2019, and 2020.

# 3.2.2. Field preparation, field design, and planting

At ShREC, the experiment was carried out in conventionally tilled field after wheat harvest. At SAREC, planting was done in no-till fallow following winter wheat (Figure 3.3). A split plot design was used at each site of the experiment (Figure 3.4). Plot sizes of each treatment in the three locations are listed in Table 3.3. Einkorn (*Triticum monococcum* L.), emmer (*Triticum turgidum* L.), spelt (*Triticum spelta* L.), and common bread wheat (*Triticum aestivum*) were grown (Table 3.4). The planting dates, and harvest dates for the study are listed in the Table 3.5. All the wheat species were sown at seeding rate of 67 kg ha<sup>-1</sup> and a seeding depth of 3.8 cm. There was cattle grazing damage on June 15<sup>th</sup>, 2020 at SAREC and the site year was lost.

#### Table 3.3. Subplot sizes of Dryland Sites

and ShREC, Sheridan, WY in spring 2019 and 2020.									
Locations		2019		2020					
	Length	Width	Area	Length	Width	Area			
	mm		m <sup>2</sup>	n	mm				
SAREC	9.1	6.4	58.2	9.1	6.1	55.5			
ShREC	6.1	6.1	37.2	6.1	6.1	37.2			

Table 3.3. Plot length, width, and area of each subplot at two study sites, SAREC, Lingle, WY and ShREC, Sheridan, WY in spring 2019 and 2020.



Figure 3.3. Planting of Dryland Sites

Figure 3.3. Planting of spelt, emmer, einkorn, and modern wheat under no-till dryland conditions in SAREC, Lingle, WY



Figure 3.4. Sample Layout of Dryland Sites

Figure 3.4. Sample layout of dryland fields at two study sites, SAREC, Lingle, WY and ShREC, Sheridan, WY in spring 2019 and 2020. A split plot design was used at each site of the experiment. There were three replications in each site (orange, blue, and green blocks). Each replication had 3 blocks, one for each nitrogen treatment (Table 3.1), giving a total of nine blocks in each site year. Nitrogen blocks were randomly allocated within each replication and was treated as the main plot. Crops (einkorn, emmer, spelt, and wheat) were randomized in subplots within the main plot.

# Table 3.4. Crop Varieties in Dryland Sites

Table 3.4. Characteristics of the spelt, emmer, einkorn, and modern wheat varieties gown at two study sites,
SAREC, Lingle, WY, PREC, and ShREC, Sheridan, WY in spring 2019 and 2020.

/	0 / /	/	,	/ 1 6		
Crop	Variety	Location	Years grown	Developing company	Maturity	Disease resistance
Spelt	CDC origin	SAREC, ShREC, PREC	2019 and 2020	University of Saskatchewan (Government of Canada, 2021)	Matures in 105-110 days	Highly resistant to loose smut and common bunt (French's hybrids, 2021)
Emmer	'Lucile'	SAREC, ShREC, PREC	2019 and 2020	Montana Foundation Seed Program (Montana Foundation Seed Program, 2003)	Heading in around 3 months (PennState, 2021)	Disease resistance is unknown (Stallknecht, 2021)
Einkorn	'Stoneage'	SAREC, ShREC, PREC	2019 and 2020	Purchased from Joel and James Starr Partnership out of Hastings, NE	It has facultative growth (spring/fall planted) (Quail seeds, 2021)	ND
Wheat	SY605 CL	SAREC	2019	Syngenta Seeds, Inc.	55 days to reach heading (WSCIA.CO, 2021).	Moderate resistance to prevalent races of leaf rust (WSCIA.CO, 2021)
	Gunnison	ShREC	2019	Westbred	Takes medium time to mature among wheat varieties	Good resistance to current races of stripe rust (WSCIA.CO, 2021)
	Surpass HRSW	SAREC	2020	North Dakota State University (NDSU)	ND	Fusarium head blight and Bacterial leaf resistance (NDSU, 2016)
	Fortuna	ShREC	2020	NDSU (Heo, et al., 2018).	Medium maturity (MSU, 2021)	Resistant to prevalent races of leaf and steam rust (WSU, 2021)

<sup>1</sup>ND means no data.

#### Table 3.5. Planting Date and Harvest Date in Dryland Sites

	Planting Date			Harvest Date							
Location				2019				2020			
	2019	2020	Einkorn	Emmer	Spelt	Control	Einl	corn	Emmer	Spelt	Control
SAREC	5-06	4-7	9-6	8-23	9-5	8-20	N	$D^1$	ND	ND	ND
ShREC	5-15	4-10	9-6	8-28	8-28	8-27	8-	11	7-30	8-11	7-30
	1 .										

Table 3.5. Planting date, and harvest date of einkorn, emmer, spelt, and modern wheat at two study sites, SAREC, Lingle, WY and ShREC, Sheridan, WY in spring 2019 and spring 2020.

 $ND^1$  means no data.

#### 3.2.3. Soil sampling for nitrogen application

Soil was sampled before planting and after harvest with a hydraulic soil probe MGSRPSUV (Giddings Machine Co, Windsor CO). At SAREC, soil was sampled in each plot at three depths (0-20 cm, 20-60 cm, 60-90 cm). At ShREC, soil sampling was done before planting and composite samples for the study area were taken at all three depths. Soil samples were analyzed at Midwest laboratories Inc., Ohama, NE. Pre-planting residual soil nitrogen at 0-20 cm soil depth was calculated. At SAREC, the average residual soil nitrogen in each block was calculated using equation 1 and at ShREC it was calculated using equation 2. Nitrogen rates of 28, 56, and 90 kg ha<sup>-1</sup> were assigned as high, medium, and low N treatments in both sites (Table 3.1). Nitrogen treatments were the sum of the residual soil nitrogen and applied soil nitrogen (eqn 3). The amount of nitrogen (N) to apply in each block was calculated using equation 4. Nitrogen was applied before planting with a tractor mounted sprayer and by using liquid nitrogen fertilizer UAN (32-0-0) in all sites.

 $\begin{aligned} Residual \ soil \ N \ at \ 0 - 20 \ cm \ depth \ of \ block \ 1 &= \frac{sum \ of \ residual \ N \ of \ four \ crop \ plots \ within \ block 1}{4} \ eqn \ (1) \\ average \ residual \ soil \ nitrogen \ in \ each \ block \ = \ residual \ soil \ N \ in \ whole \ study \ site \ eqn \ (2) \\ Nitrogen \ treatment \ = \ Residual \ soil \ nitrogen \ + \ applied \ soil \ nitrogen \ eqn \ (3) \\ Nitrogen \ (N) \ application \ in \ block 1 \end{aligned}$
#### 3.2.4. Gravimetric water balance procedure

Soil was sampled before planting and after harvest with a hydraulic soil probe MGSRPSUV (Giddings Machine Co, Windsor CO) at SAREC and ShREC. A 3.8 cm core diameter soil was collected in each treatment plot at three depths (0-20 cm, 20 cm-60 cm, 60-90 cm) for preplanting sampling at SAREC, post-harvest sampling at SAREC, and post-harvest sampling at ShREC. For pre-planting sampling at ShREC, a composite 3.8 cm core diameter soil sample representing whole study site was taken at the same three depths. The samples were weighed then dried at a 105°C for at least 48 hours then reweighed to determine the gravimetric moisture content.

## **3.2.5.** Data collection

#### 3.2.5.1. Stand counts

In each plot, the number of plants germinated in three one-meter rows were counted and the average number of plants  $m^{-2}$  was calculated. Three rows in each plot were selected in such a way that they made a diagonal across the plots. Sampled area (eqn 5) was used to calculate plant population (eqn 6).

Sampled area 
$$(m^2) = 1 (m) * row width (m)$$
 eqn (5)

Population per 
$$m^2 = \frac{average number of plants in sampled area}{sampled area (m^2)}$$
 eqn (6)

#### 3.2.5.2. NDVI

NDVI readings were taken on a weekly interval at SAREC dryland by using RapidSCAN CS-45 Holland scientific handheld crop sensor (Holland Scientific Inc, Lincoln NE; Figure 3.5). NDVI unit was held about one meter above the plant canopy and data was recorded by moving across the same row throughout the growing period.



Figure 3.5. Monitoring Crop Growth with NDVI

Figure 3.5. Taking NDVI (Normalized Difference Vegetation Index) to monitor the crop growth over time. NDVI was taken on weekly interval at SAREC by using RapidSCAN CS-45 Holland scientific handheld crop sensor (Holland Scientific Inc, Lincoln NE)

# 3.2.5.3. Plant stage

The Feekes growth scale (Table 3.6; Wise et al., 2011) was used to evaluate the growth stage of

all the ancient wheats and the control wheat weekly at SAREC. Only heading, and harvest date

was recorded at ShREC.

# 3.2.5.4. Lodging

Lodging is when the crop falls over which largely affects yield if it occurs after anthesis

(CIMMYT, 2021). Lodging was measured by using Horsfall-Barratt disease scoring scale

(Francis, 2019; Table 3.7). Percentage of infection was modified to percentage of lodging and

disease rating was modified to lodging score in that scale (Table 3.7)

# Table 3.6. Feekes Growth Scale

Feekes	Common Stage	Characteristics
Scale	Name	
1	Emergence	Most of the seedlings are emerged.
2-3	Tillering	Plant develops tillers. Tillers are auxiliary or side shoots
4	Green up	Plant starts to have erect growth.
5	Green up	Plant leaf sheath lengthens.
6	Jointing	Plant develops the first node at the base of the shoot.
7	Two nodes	Two nodes of the plant are visible above the soil line.
8	Flag leaf	Flag leaf appears.
9	Flag leaf ligule	Flag leaf ligule becomes visible.
10	Boot stage	Wheat head is visible inside the swollen leaf sheath.
10.5	Heading	The complete head comes out of the boot
10.5.1	Flowering	Flowering begins
10.5.3	Pollination	Pollination is complete
10.5.4	Watery ripe	Watery ripe of kernels
11.1	Milky ripe	Milky ripe of kernels
11.2	Soft dough	Kernels have doughy or mealy consistency
11.3	Hard dough	Kernels are hardened
11.4	Harvest	Ready to harvest

Table 3.6. Details of Feekes growth scale (Wise et al., 2011) used to record growth stages at SAREC in spring 2019 and 2020.

# Table 3.7. Horsfall-Barratt Scale

Table 3.7. Horsfall-Barratt scale disease scoring scale modified to score lodging at SAREC, Lingle, WY and ShREC, Sheridan, WY in spring 2019, and 2020. Percentage of infection was modified to percentage of lodging and disease rating was modified to lodging score (Francis, 2019).

Score	Percent Lodged
	%%
1	0
2	0-3
3	3-6
4	6-12
5	12-25
6	25-50
7	50-75
8	75-87
9	87-94
10	94-97
11	97-100
12	100

### 3.2.5.5. Yield and yield parameters

Heading height from the ground to the top of the head of three plants per plot was measured at ShREC. Grain samples from the center of each plot were harvested with a small plot combine. Samples were cleaned to remove chaff, weighed, and test weight was measured by using USDA test weight apparatus. Each plot length was measured before harvesting and the sampled area in hectare was calculated. Yield in kg ha<sup>-1</sup> was calculated by:

$$Yield (kg ha^{-1}) = \frac{Yield (kg)}{Area (ha)}$$
eqn (7)

Ancient wheats are hulled and need extra dehulling process. Hulled grain samples were dehulled using a Kimseed thresher (Kimseed Australia, Wangara WA; Figure 3.6) and cleaned by using laboratory thresher Haldrup LT-35 (Haldrup USA, Ossian IN; Figure 3.6) and soil sieves. Grain weight and grain test weight were measured, and grain yield (kg ha<sup>-1</sup>) was calculated. Percent yield loss to the hull was calculated as;

Yield loss to hull (%) = 
$$\left[1 - \frac{grain \ yield}{hulled \ yield}\right] \times 100\%$$
 eqn (8)

Nitrogen use efficiency (NUE), which is the fraction of applied nitrogen that is absorbed and used by the plant (UCDAVIS, 2021) was calculated as;

 $NUE = \frac{Grain \ yield}{Residual \ soil \ nitrogen + applied \ nitrogen}$ (Moll, Kamprath, & Jackson, 1982; Appendix 4)

eqn (9)



Figure 3.6. Dehulling and Cleaning of Ancient Wheats

Figure 3.6. Dehulling and cleaning of ancient wheats, spelt, emmer, and einkorn, using a Kimseed thresher (Kimseed Australia, Wangara WA) and laboratory thresher Haldrup LT-35 (Haldrup USA, Ossian IN) respectively.

# 3.2.5.6. Grain quality

The dehulled and cleaned ancient grain samples were further cleaned using different size soil sieves and a laboratory thresher (Model LT-35, Haldrup). For each plot in each site, 50 g full size naked grains were obtained by removing dirt, hulls, and broken grains. Then grains of each treatment from three replications were combined to make a composite 150 g sample for each treatment (nitrogen x crop x location) and sent to the California Wheat Commission Lab (California Wheat Commission, Woodland CA) for protein analysis by combustion method. Grain protein was compared for each treatment. Total grain nitrogen yield was calculated by;

Grain nitrogen yield = 
$$\frac{Protein}{5.7}$$
 (Gauer et al., 1992) eqn (10)

Total grain nitrogen yield  $(kg ha^{-1}) = grain nitrogen yield \times yield (kg ha^{-1}) eqn (11)$ 

### 3.2.5.7. Grasshopper damage

There was grasshopper damage in the dryland field at SAREC on 30<sup>th</sup> July 2019 (Appendix 2). Ten random flag leaves plot<sup>-1</sup> were taken and assessed for feeding damage (Appendix 2). For damage severity, each plot was evaluated by Horsfall-Barrat scale (Table 3.5; Francis, 2019).

#### 3.2.5.8. Grazing damage

Grazing damage was observed in SAREC on June 16<sup>th</sup>, 2020. Horsefall-Barrat scale (Table 3.5; Francis, 2019) was used to score grazing damage. Grazing resulted in complete loss of the study in 2020.

3.2.5.9. Water use and water use efficiency

Water use and water use efficiency of ancient wheats was studied at SAREC, and ShREC in spring 2019. SAREC spring 2020 plots were lost due to grazing damage. Water use and water use efficiency at ShREC in 2020 was not tested due to covid19 travel restrictions. Gravimetric water content, soil volume, bulk density, volumetric water content, water use, and water use efficiency were determined for each treatment using equations 12-17.

Volume of soil = 
$$\pi r^2 h$$
 (r = radius of soil core; h = depth of core) eqn (12)

$$Bulk \ density = \frac{Dry \ weight \ of \ soil}{Volume \ of \ soil} (Bilskie, 2021)$$
eqn (13)

Gravimetric water content (
$$\theta g$$
) =  $\frac{Wet weight of soil - Dry weight of soil}{Dry weight of soil}$  (Bilskie, 2021) eqn (14)

*Volumetric water content* = 
$$\theta_g \times Bulk$$
 *density* (Gan et al., 2007) eqn (15)

Water use 0 - 90 cm depth = Volumetric water content at preplanting + precipitation volumetric water content at postharvest (Gan et al., 2007) eqn (16)

Water use efficiency at 
$$0 - 90 \text{ cm depth} = \frac{Grain \text{ yield}}{Water \text{ use}}$$
 (Gan et al., 2007) eqn (17)

#### **3.2.6.** Data analysis

Linear mixed effect models were run separately for each species using lme4 package (Bates et al., 2015) in the R statistical language (v 3.5.1) (RStudio Team, 2020) with location, nitrogen, and the interaction between location and nitrogen as fixed factors, and year as a random factor on crop growth and yield parameters. The model is illustrated in the eqn (18) where Y<sub>ij</sub> is the response for i<sup>th</sup> parameter and j<sup>th</sup> crop, location is the site where study was conducted, nitrogen is the starting soil nitrogen (eqn 3), nitrogen\*location is the interaction between nitrogen and location. Location, nitrogen, and interaction between location and nitrogen were treated as fixed factor and year was treated as random factor in the model. For NDVI, location was removed as there was data for only one location.

To quantify the effect of crop species on crop growth and yield parameters a mixed linear model was run for each location separately where crop is the fixed factor and year is random factor in the model. The model is illustrated in eq<sup>n</sup> (19) where  $Y_{ij}$  is the response for i<sup>th</sup> parameter and j<sup>th</sup> location, crop is the wheat species.

$$Y_{ij} \sim crop + |year|$$
 eqn (19)

Type III Analysis of Variance were extracted for all models. Where appropriate, logtransformation was applied to the response variable to achieve homogeneity of variances. Tukeyadjusted pairwise treatment comparisons were performed using the "emmeans" package at 5% significance level (Searle et al., 1980).

# 3.3. Results

# 3.3.1. Crop growth

## 3.3.1.1. Effect of nitrogen and location on crop growth

The effect of nitrogen treatment and growing location on plant stand, lodging, heading height, and days to heading was tested for each crop (Table 3.8). For all crops nitrogen and the nitrogen by location interaction had no effect on plant stand, lodging, heading height, and days to heading. Location significantly affected lodging of all species, plant stands of all species except einkorn, and heading days of all species except modern wheat (Table 3.8). All the crops had a longer heading period and lower lodging at SAREC than at ShREC (Table 3.9). Plant stands of emmer and spelt was higher at SAREC than at ShREC whereas plant stands of wheat and einkorn were higher at ShREC than at SAREC (Table 3.9).

# Table 3.8. Effect of Nitrogen and Location on Dryland Crop Growth

Table 3.8. Analysis of variance showing P-Values for the effect of nitrogen, location, and interaction between nitrogen and location on plant stands, lodging score (Table 3.7), heading height, and heading days of different spring wheat species in 2019 and 2020 at dryland fields of two University of Wyoming research stations, SAREC, Lingle, WY, ShREC, Sheridan, WY. Analysis was done separately for each crop. Heading height was not recorded at SAREC.

Crop	Factor	Plant	Lodging	Heading	Heading
		Stands	Score	Height	Days
		Plants m <sup>-2</sup>		-cm-	
Einkorn	Location	0.78	0.002	$NA^1$	< 0.001
	Nitrogen	0.98	0.5	0.89	0.96
	Location:nitrogen	0.07	0.37	NA	0.96
Emmer	Location	< 0.001	0.03	NA	< 0.001
	Nitrogen	0.64	0.73	0.37	1
	Location:nitrogen	0.44	0.73	$NA^1$	1
Spelt	Location	< 0.001	< 0.001	NA	< 0.001
	Nitrogen	0.99	0.97	0.28	0.96
	Location:nitrogen	0.51	0.97	NA	0.96
Wheat	Location	< 0.001	0.017	NA	0.12
	Nitrogen	0.95	0.61	0.44	1
	Location:nitrogen	0.77	0.61	NA	1

P-Value <0.05 are significant at 5% significance level. <sup>1</sup>NA means not applicable.

## Table 3.9. Dryland Crop Growth Comparison by Location

Table 3.9. Pairwise comparison for the effect of location on plant stands, lodging score (Table 3.7), heading height, and heading days of different spring wheat species in 2019 and 2020 at dryland fields of two University of Wyoming research stations, SAREC, Lingle, WY and ShREC, Sheridan, WY.

Crop	Location	Plant Stands	Lodging Score	Heading Days
		Plants m <sup>-2</sup>		
Einkorn	SAREC	$NS^1$	1.0 B	91.4 A
	ShREC	NS	1.3 A	73.5 B
Emmer	SAREC	157.0 A	1.0 B	74.0 A
	ShREC	116.0 B	1.2 A	66.5 B
Spelt	SAREC	127.5 A	1.0 B	81.0 A
	ShREC	71.9 B	1.5 A	69.5 B
Wheat	SAREC	178.0 B	1.0 B	$NS^1$
	ShREC	251.0 A	1.7 A	NS

Within column means followed by the same uppercase letters are not different at  $\alpha$ =0.05. <sup>1</sup>NS means not significant.

### 3.3.1.2. Effect of wheat species on crop growth

Within each location, wheat species had a significant effect on plant stand, heading height, and days to heading (Table 3.10). The plant stand of wheat was the highest, followed by einkorn, and emmer, and then the spelt in both locations (Table 3.11). There was no lodging at SAREC. At ShREC, lodging was very low and similar among the crops. Heading height of all ancient wheats was higher than modern wheat at ShREC (Table 3.11). The heading height of spelt was higher than einkorn but the difference was less than 10 cm (Table 3.11). Days to heading were longest for the einkorn, followed by spelt, emmer, and then modern wheat in both locations (Table 3.11).

### Table 3.10. Effect of Wheat Species on Dryland Crop Growth

Table 3.10. Analysis of variance showing P-Values for the fixed effect of wheat species on plant stands, lodging score (Table 3.7), heading height, and heading days at dryland fields of two University of Wyoming research stations, SAREC, Lingle, WY and ShREC, Sheridan, WY in spring 2019 and 2020.

Location	Factor	Plant Stands	Lodging Score	Heading Height	Heading Days
		Plants m <sup>-2</sup>	Score	cm	
SAREC	crop	< 0.001	$\mathbf{NL}^1$	$ND^2$	< 0.001
ShREC	crop	< 0.001	0.22	< 0.001	< 0.001

P-Value <0.05 are significant at 5% significance level. <sup>1</sup>NL means no lodging. <sup>2</sup>ND means no data.

#### Table 3.11. Dryland Crop Growth Comparison by Location

Table 3.11. Pairwise comparison for the effect of wheat species on plant stands, heading height, and heading days at dryland fields of two University of Wyoming research stations, SAREC, Lingle, WY and ShREC, Sheridan, WY in spring 2019 and 2020.

Location	Crop	Plant Stands	Heading Height	Heading Days
		Plants m <sup>-2</sup>	-cm-	
SAREC	Einkorn	154.0 A	$ND^1$	91.3 A
	Emmer	157.0 A	ND	74.0 C
	Spelt	128.0 B	ND	81.0 B
	Wheat	178.0 A	ND	64.0 D
ShREC	Einkorn	156.5 B	85.8 B	73.5 A
	Emmer	125 C	92.2 AB	66.5 C
	Spelt	71.9 D	93.8 A	69.5 B
	Wheat	251.5 A	70.6 C	61.5 D

Within columns, means followed by the same uppercase letters are not different at  $\alpha$ =0.05. <sup>1</sup>ND means no data.

### 3.3.2. NDVI

3.3.2.1. Effect of nitrogen on NDVI at SAREC:

The effect of nitrogen treatment on NDVI was tested for each crop at tillering, jointing, flag leaf,

and heading stage (Table 3.12). Nitrogen had no effect on NDVI for all crops at all stages.

# Table 3.12. Effect of Nitrogen on Dryland NDVI

Table 3.12. Analysis of variance showing P-Values for the fixed effect of nitrogen on NDVI of different spring wheat species in 2019 and 2020 at the dryland fields of SAREC, Lingle, WY. Crops were in different stages on the same date. The p-value in the table represent the data from several dates even within same plant stage.

Crop	Factor	Tillering	Jointing	Flag leaf	Heading
Einkorn	Nitrogen	0.4	0.87	0.81	0.16
Emmer	Nitrogen	0.8	0.79	0.72	0.63
Spelt	Nitrogen	0.92	0.45	0.19	0.80
Wheat	Nitrogen	0.78	0.30	0.19	0.58

P-Value <0.05 are significant at 5% significance level.

# 3.3.2.2. Effect of wheat species on NDVI at SAREC:

Wheat species had significant effect on NDVI at jointing, flag leaf, and heading stages (Table 3.13). Einkorn had the highest NDVI, followed by spelt, emmer, and then modern wheat at flag leaf, and jointing stages (Table 3.14). At heading, spelt had the highest NDVI and rest of the crops had similar NDVI (Table 3.14). Ancient wheats had higher NDVI than modern wheat at jointing and flag leaf stages suggesting a higher vegetative growth at those stages and potential to grow as forage crops than modern wheat (Table 3.14). Einkorn had higher NDVI than spelt at vegetative growth stages (jointing and flag leaf) but lesser in reproductive period (heading). NDVI of emmer, spelt, and modern wheat increased on progressing from jointing to flag leaf to heading (Table 3.14). NDVI of einkorn increased on progressing from jointing to flag leaf and then decreased when it reached heading (Table 3.14).

## Table 3.13. Effect of Wheat Species on Dryland NDVI

Table 3.13. Analysis of variance showing P-Values for the fixed effect of wheat species on NDVI at SAREC, Lingle, WY in spring 2019 and 2020. P-Value reported at heading stage is from spring 2019. Spring 2020 was lost after flag leaf stage due to grazing damage.

Factor	Tillering	Jointing	Flag leaf	Heading
Crop	0.1	< 0.001	< 0.001	< 0.001

P-Value <0.05 are significant at 5% significance level.

## Table 3.14. Dryland NDVI Comparison by Wheat Species

from spring 2019. Spring 2020 was lost after flag leaf stage due to grazing damage.						
Crop	Jointing	Flag leaf	Heading			
Einkorn	0.37 A	0.50 A	0.44 B			
Emmer	0.28 B	0.40 B	0.42 B			
Spelt	0.28 B	0.43 AB	0.53 A			
Wheat	0.22 C	0.26 C	0.45 B			

Table 3.14. Pairwise comparison for the effect of wheat species on NDVI at the dryland fields of SAREC, Lingle, WY in spring 2019 and 2020. NDVI reported at heading stage is from spring 2019. Spring 2020 was lost after flag leaf stage due to grazing damage.

Within columns, means followed by the same uppercase letters are not different at  $\alpha$ =0.05.

## **3.3.3.** Yield parameters

## 3.3.3.1. Response of yield and quality parameters to nitrogen, and location:

The effect of nitrogen treatment and growing location on hulled yield, grain yield, hull loss, hulled test weight, grain test weight, grain protein %, total grain N yield (kg ha<sup>-1</sup>) was tested for each crop (Table 3.15). For all crops nitrogen and the nitrogen by location interaction had no effect on yield and quality parameters. Location significantly affected hulled yield of all ancient wheats, grain yield of emmer, spelt, and modern wheat, yield loss to hull for einkorn and emmer, hulled test weight of einkorn, and spelt, protein % of einkorn, emmer, and wheat, and total grain N yield of emmer, spelt, and modern wheat (Table 3.15). SAREC 2020 plots were lost due to grazing damage. Grain yield, hulled yield, protein, and total grain N yield of all the crops were higher at ShREC than at SAREC (Table 3.16). Grain test weight of all crops was similar in both locations whereas hulled test weight of einkorn and spelt was higher at SAREC for emmer, and similar in both locations for spelt (Table 3.16).

## Table 3.15. Effect of Nitrogen and Location on Yield and Quality

Table 3.15. Analysis of variance showing P-Values for the fixed effect of location, nitrogen, and interaction between location and nitrogen on hulled yield, grain yield, loss, hulled test weight, grain test weight, grain protein, and total grain N yield of different spring wheat species during spring 2019 and 2020 at dryland fields of two University of Wyoming research stations; SAREC, Lingle, WY and ShREC, Sheridan, WY. Values reported in SAREC are for spring 2019. In 2020, plots at SAREC were lost due to grazing damage.

Parameter	Factor	Einkorn	Emmer	Spelt	Wheat
Hulled Yield			Kg ha <sup>-1</sup> -		
	Location	0.008	0.02	< 0.001	$NA^1$
	Nitrogen	0.98	0.76	0.65	NA
	Location:nitrogen	0.99	0.51	0.87	NA
Grain Yield	Kg ha <sup>-1</sup>				
	Location	0.2	0.02	< 0.001	0.0011
	Nitrogen	0.99	0.78	0.87	0.66
	Location:nitrogen	0.93	0.68	0.96	0.61
Loss			%		
	Location	< 0.001	0.011	0.95	NA
	Nitrogen	0.45	0.45	0.058	NA
	Location:nitrogen	0.22	0.75	0.1	NA
Hulled Test			Kg/bushe	l	
Weight	Location	< 0.001	0.49	< 0.001	NA
	Nitrogen	0.96	0.92	0.83	NA
	Location:nitrogen	0.39	0.51	0.98	NA
Grain Test			ŀ	Kg/bushel	
Weight	Location	0.11	0.18	0.329	0.78
	Nitrogen	0.69	0.57	0.325	0.54
	Location:nitrogen	0.051	0.82	0.09	0.88
Protein				%	
	Location	0.0016	0.03	0.057	0.03
	Nitrogen	0.44	0.52	0.43	0.64
	Location:nitrogen	0.5	0.57	0.82	0.89
Total Grain N			ŀ	Kg ha <sup>-1</sup>	
Yield	Location	0.11	0.0014	< 0.001	< 0.001
	Nitrogen	0.99	0.63	0.68	0.73
	Location:nitrogen	0.96	0.79	0.88	0.65

P-Value <0.05 are significant at 5% significance level. <sup>1</sup>NA means not applicable.

### Table 3.16. Dryland Yield and Quality Comparison by Location

Table 3.16. Pairwise comparison for the effect of location on hulled yield, grain yield, loss,
hulled test weight, grain test weight, grain protein, and total grain N yield at dryland fields
of two University of Wyoming research stations; SAREC, Lingle, WY and ShREC,
Sheridan, WY in spring 2019 and 2020. Values reported in SAREC are for spring 2019.

Parameter	Location	Einkorn	Emmer	Spelt	Wheat	
Hulled Yield	Kg ha <sup>-1</sup>					
	ShREC	1151.0 A	1063.0 A	1459.0 A	$NA^1$	
	SAREC	672.0 B	1252.0 B	629.0 B	NA	
Grain Yield			Kg	ha <sup>-1</sup>		
	ShREC	611.2 A	1122.0 A	901.0 A	2196.0 A	
	SAREC	156.6 A	825.0 B	387.0 B	1238.0 B	
Loss			%			
	ShREC	51.5 A	30.7 B	38.1 A	NA	
	SAREC	31.1 B	37.0 A	40.7 A	NA	
Hulled Test			Kg/bush	el		
Weight	ShREC	12.1 B	15.8 A	12.4 B	NA	
	SAREC	13.9 A	15.0 A	14.2 A	NA	
Grain Test			Kg/t	oushel		
Weight	ShREC	26.5 A	26.9 A	27.4 A	28.2 A	
	SAREC	26.3 A	26.2 A	27.3 A	27.7 A	
Protein				%		
	ShREC	18.5 A	16.2 A	16.9 A	15.8 A	
	SAREC	15.4 B	13.5 B	15.7 A	13.2 B	
Total Grain N			Kg	ha <sup>-1</sup>		
Yield	ShREC	19.8 A	31.6 A	26.6 A	59.6 A	
	SAREC	4.2 A	18.8 B	10.2 B	26.0 B	

Within columns, means followed by the same uppercase letters are not different at  $\alpha$ =0.05. <sup>1</sup>NA means not applicable.

#### 3.3.3.2. Response of yield and quality parameters to wheat species:

Within each location, grain species had significant effect on hulled yield, grain yield, yield loss to hull, hulled test weight, grain test weight, grain protein %, total grain N yield (kg ha <sup>-1</sup>) except yield loss to hull at SAREC (Table 3.17). Hulled yield and hulled test weight were higher for emmer, followed by spelt, and then einkorn in both locations. Grain yield was higher for wheat, followed by emmer, spelt, and then einkorn in both locations (Table 3.18). Grain test weight was

higher for modern wheat and spelt than einkorn, and emmer in both locations. Protein was the highest for einkorn at ShREC. Spelt had the highest protein content followed by einkorn, modern wheat, and then emmer at SAREC. Total grain N yield were the highest for modern wheat, followed by emmer, spelt, and then einkorn in both locations (Table 3.18).

## Table 3.17. Effect of Wheat Species on Dryland Yield and Quality

Table 3.17. Analysis of variance showing P-Values for the fixed effect of wheat species on hulled yield, grain yield, loss, hulled test weight, grain test weight, grain protein, and total grain N yield at dryland fields of two University of Wyoming research stations; SAREC, Lingle, WY and ShREC, Sheridan, WY in spring 2019 and 2020.

Parameter	Factor	ShREC	SAREC		
Hulled Yield		Kg ha <sup>-1</sup>			
	Crop	0.003	< 0.001		
Grain Yield		Kg	; ha <sup>-1</sup>		
	Crop	< 0.001	< 0.001		
Loss		0	%		
	Crop	< 0.001	0.29		
Hulled Test Weight		Kg/	bushel		
	Crop	< 0.001	< 0.001		
Grain Test Weight		Kg/bushel			
	Crop	< 0.001	< 0.001		
		%%			
Protein	Crop	< 0.001	0.002		
Total Grain N Yield		Kg ha <sup>-1</sup>			
	Crop	< 0.001	< 0.001		

P-Value <0.05 are significant at 5% significance level.

#### Table 3.18. Dryland Yield and Quality Comparison by Location

Table 3.18. Pairwise comparison for the fixed effect of wheat species on hulled yield, grain yield, loss, hulled test weight, grain test weight, grain protein, and total grain N yield at dryland fields of two University of Wyoming research stations, SAREC, Lingle, WY and ShREC, Sheridan, WY in spring 2019 and 2020.

Location	Crop	ShREC	SAREC
Hulled Yield		K	g ha <sup>-1</sup>
	Einkorn	1151 B	283 C
	Emmer	1612 A	1139 A
	Spelt	1454AB	629 B
	Wheat	NA	NA
Grain Yield		Kg	g ha -1
	Einkorn	611 C	157 D
	Emmer	1135 B	695 B
	Spelt	901 BC	371 C
	Wheat	2196 A	875A
Loss			%
	Einkorn	51.5 A	$NS^2$
	Emmer	30.3 C	NS
	Spelt	38.1 B	NS
	Wheat	NA	NS
Hulled Test Weight		Kg	/bushel
C	Einkorn	12.1 B	13.5 B
	Emmer	15.9 A	15.0 A
	Spelt	12.4 B	13.0 B
	Wheat	NA	NA
Grain Test Weight		Kø	/bushel
Gruin Test Weight	Finkorn	26 5 C	26 3 B
	Emmer	26.9 C	26.3 B
	Spelt	20.9 C	20.2 D
	Wheat	27.4 D 28.2 A	27.3A
	w neat	20.2 A	0/
Drotain	Finleson	 10 5 A	70 15 / AD
FIOLEIII	EIIIKOIII	10.3 A	13.4 AB
	Emmer	10.2 B	14.3 C
	Spelt	10.9 B	15./ A
	Wheat	15.8 B	14.6 BC
Total Grain N Yield		Kg	g ha -1
	Einkorn	19.8 C	4.2 D
	Emmer	32.0 B	17.5 B
	Spelt	26.6 BC	10.2 C
	Wheat	59.6 A	22.3 A

Within columns, means followed by the same uppercase letters are not different at  $\alpha$ =0.05. <sup>1</sup>NA means not applicable. <sup>2</sup>NS means not significant.

## **3.3.4.** Water use and water use efficiency

## 3.3.4.1. Response of water use and water use efficiency to nitrogen, and location:

The effect of nitrogen treatment and growing location on water use (mm) and water use efficiency (kg ha<sup>-1</sup> mm<sup>-1</sup>) was tested for each crop in spring 2019 (Table 3.19). For all crops nitrogen and the nitrogen by location interaction had no effect on water use and water use efficiency. Location significantly affected water use and water use efficiency of all the ancient and modern wheat (Table 3.19). Water use for all crops was higher at SAREC than at ShREC (Table 3.20). Water use efficiency for all crops was higher at ShREC than at SAREC (Table 3.20).

### 3.3.4.2. Response of water use and water use efficiency (WUE) to wheat species:

Within each location, wheat species had significant effect on water use efficiency but not the water used (Table 3.21). Water use efficiency was the highest for modern wheat, followed by emmer, spelt, and then einkorn in both locations (Table 3.22).

### **Table 3.19.** Effect of Nitrogen and Location on Dryland Water Use Efficiency

Table 3.19. Analysis of variance showing P-Values for the fixed effect of location,								
nitrogen, and interaction between location and nitrogen on water use and water use								
efficiency of different wheat species in spring 2019 at dryland fields of two University								
of Wyoming	g research stations, SA	REC, Lingle,	WY and ShR	EC, Sheridan,	WY.			
Parameter	er Factor Einkorn Emmer Spelt Wheat							
Water	mm							
Use	Location	< 0.001	< 0.001	< 0.001	< 0.001			
	Nitrogen	0.08	0.48	0.99	0.81			
	Location:nitrogen	0.16	0.82	0.19	0.16			
Water	kg ha <sup>-1</sup> mm <sup>-1</sup>							
Use	location	0.009	< 0.001	< 0.001	< 0.001			
Efficiency	nitrogen	0.57	0.29	0.5	0.1			
	location:nitrogen	0.66	0.78	0.6	0.09			

1 1 0 1 66 0.1

P-Value <0.05 are significant at 5% significance level.

## Table 3.20. Dryland Water Use Efficiency Comparison by Location

SAREC, Lingle, wY and ShREC, Sheridan, wY in spring 2019.						
Parameter Location		Einkorn	Emmer	Spelt	Wheat	
Water Use		mm				
	SAREC	334.9 A	334.9 A	334.9 A	334.9 A	
	ShREC	238.4 B	238.4 B	238.5 B	238.4 B	
Water Use	Water Usekg ha <sup>-1</sup> mm <sup>-1</sup>					
Efficiency	SAREC	0.5 B	2.1 B	1.1 B	2.6 B	
	ShREC	1.1 A	4.1 A	3.6 A	7.5 A	

Table 3.20. Pairwise comparison for the fixed effect of location on water use and water use efficiency at dryland fields of two University of Wyoming research stations, SAREC Lingle WY and ShREC Sheridan WY in spring 2019

Within columns, means followed by the same uppercase letters are not different at  $\alpha$ =0.05.

### Table 3.21. Effect of Wheat Species on Water Use Efficiency

Table 3.21. Analysis of variance showing P-Values for the fixed effect of wheat species on water use, and water use efficiency at dryland fields of two University of Wyoming research stations; SAREC, Lingle, WY and ShREC, Sheridan, WY in spring 2019.

Parameter	Factor	ShREC	SAREC		
Water use		mm			
	Crop	0.12	0.06		
WUE		kg ha <sup>-1</sup> mm <sup>-1</sup>			
	Crop	< 0.001	< 0.001		

P-Value <0.05 are significant at 5% significance level.

### Table 3.22. Dryland Water Use Efficiency Comparison by Location

Table 3.22. Pairwise comparison for the fixed effect of wheat species on water use efficiency at dryland fields of two University of Wyoming research stations; SAREC, Lingle, WY and ShREC, Sheridan, WY in spring 2019.

Location	Crop	ShREC	SAREC		
WUE		kg	kg ha <sup>-1</sup> mm <sup>-1</sup>		
	Einkorn	1.1 C	0.5 D		
	Emmer	4.1 B	2.1 B		
	Spelt	3.6 B	1.1 C		
	Wheat	7.5 A	2.6 A		

Within columns, means followed by the same uppercase letters are not different at  $\alpha$ =0.05.

#### **3.4. Discussion**

Einkorn, emmer, and spelt are believed to be suited for production in marginal low input system (Troccoli & Codianni, 2005) and may be potential alternative crops for dryland fields of Wyoming. We measured the agronomic and quality performance of ancient wheats in dryland fields under three nitrogen treatments and two Wyoming growing locations. In our study, nitrogen treatment had no effect on growth, yield, and quality parameters of ancient wheats and modern grains. Location affected the growth, yield, and quality parameters of ancient wheat species and einkorn, emmer, and spelt had unique growth, yield, and quality parameters within each location.

#### 3.4.1. Nitrogen treatment had no effect on growth, yield, quality, and WUE

Nitrogen had no effect on the plant stands, lodging, heading days, yield, test weight, protein, total grain nitrogen yield, water use, and water use efficiency of ancient and modern wheat under dryland conditions (Table 3.8, 3.15 and 3.19). Walsh, (2019) reported a similar finding in which grain yield of spring wheat grown in no tilled dryland was not affected by increasing nitrogen rate from 0-270 kg ha<sup>-1</sup> in one location. Alemu & Bayisa (2016) even reported that emmer had the highest yield at the low nitrogen rate (0-23 kg ha<sup>-1</sup>) compared to 46-69 kg ha<sup>-1</sup>.

However, Castagna et al. (1996) reported that split nitrogen application totaling 50 kg ha<sup>-1</sup> vs no application increased the yield of modern wheat but not ancient wheat. Marino et al. (2009) found that the yield of emmer increased by 175% at 90 kg ha<sup>-1</sup> split nitrogen application, 140% at 60 kg ha<sup>-1</sup> split nitrogen application, and 80% at 30 kg ha<sup>-1</sup> split nitrogen application compared to no nitrogen application. Nitrogen was split at seeding, tillering, and stem elongation in their study. These findings suggest that at least modern wheat should have shown some response to nitrogen application in our study. The loss of applied nitrogen could be the reason for no

nitrogen response in our study. Very low precipitation in our growing regions (Figure 3.2) compared to the above studies may be one of the reasons for no nitrogen response in our study. Average precipitation at the study site of Marino et al. (2009) was 727 mm. In water-limited ecosystems, nitrogen uptake from the soil is limited by water availability and overall plant productivity decreases (Cregger et al., 2014). Nitrogen application was done pre-planting by sprayer without soil incorporation using liquid urea ammonium nitrate (UAN). Surface applied ammonia- and ammonium-based nitrogen fertilizer are susceptible to nitrogen loss by ammonia volatilization (Jones, Brown, Engel, Horneck, & Olson-Rutz, 2020). We used urease inhibitor to control ammonium loss. But it restricts urease hydrolysis only up to 7-14 days (IPNI, 2021). So, our applied nitrogen was susceptible to loss 14 days after application. Soil temperature was also low (5-9°C in Lingle in 2019 and 2020, 4°C in ShREC in 2020) (WACNet, 2021) when we applied nitrogen. We do not have soil temperature for ShREC in 2019. But we assume that the soil temperature at SHREC in 2019 was lower than/similar to the soil temperature in 2020 as the air temperature in 2019 was lower than air temperature in 2020 (Figure 3.2). At low soil temperature microbial activity decreases hampering the ammonization and nitrification process and ultimately decreasing the amount of available nitrogen to plants (Havlin, Tisdale, Nelson, & Beaton, 2013). Incorporating nitrogen source in soil, late planting to avoid low soil temperature and irrigation can help to reduce nitrogen loss and obtain benefit of nitrogen addition. Split nitrogen application in the above studies compared to pre-planting one time application in our study can be another reason for contrasting results. Split application of nitrogen can potentially reduce nitrogen losses and lead to a better translocation of pre-anthesis assimilates to the grain (Abdin et al., 1996). Some studies have demonstrated potential benefits of nitrogen splitting in various growth stages of ancient wheats. Marino et al. (2011) found that emmer yield increased

on increasing nitrogen application and yield was the highest when 90 kg ha<sup>-1</sup> nitrogen was applied one time at tillering stage or split as 30-30-30 in seeding, stem elongation and tillering. Grain protein in our study ranged from 14-18% even at low nitrogen treatment. This protein content is similar to protein content observed by Castagna et al. (1996) and Marino et al. (2009). Cazzato et al. (2013) reported a similar finding in which emmer, and spelt had considerable forage yield, and quality even at low nitrogen. This supports our finding that the ancient and modern wheat were able to maintain quality even at low nitrogen application. However, due to no yield response to nitrogen application, studies using higher dose of nitrogen and applying measures to reduce nitrogen loss such as soil nitrogen incorporation, split application at various stages is suggested for Wyoming production.

## 3.4.2. Location affected the growth, yield, quality, and WUE

Location had significant effect on growth, yield, and quality parameters of ancient and modern wheat (Table 3.8 and 3.15). Plant stands and water use was lower, and lodging was higher at ShREC than at SAREC (Table 3.9). ShREC was planted in tilled fields whereas SAREC was planted in no-tilled fields with crop residues. Less water is lost to the atmosphere in field with crop residue vs conventionally tilled field. This might be the reason for higher plant stands and lower water use at SAREC than at ShREC. Lodging in cereals can occur due to several other factors like high nitrogen level, wet soil, direction of wind flow, and poor straw strength (Ransom, 2015). Any of these factors might be the reason for higher lodging at ShREC than at SAREC. Days to heading was longer at SAREC than at ShREC (Table 3.9). Wheat crops need to accumulate a certain number of growing degree day to reach different growth stages (NDAWN, 2021). SAREC reached the same GDD as ShREC later in the season (Table 3.23) which was likely the reason for longer heading period at SAREC than at ShREC. Average grain yield, grain

protein, total grain nitrogen yield, and water use efficiency of all the wheat species were higher at ShREC in spring 2019 and 2020 than at SAREC in spring 2019 (Table 3.16). In 2020, plots at SAREC were lost due to grazing damage. Growing degree day from heading to harvest does not seem to be different in two locations (Table 3.23). Thus, GDD did not affect the performance across two locations. The difference in land preparation method could have affected the performance of two locations. SAREC was planted in no tilled field with crop residues whereas ShREC was planted in conventionally tilled field. Crop residues in soil increase nitrogen volatilization and immobilization (Jones, Brown, Engel, Horneck, & Olson-Rutz, 2020; Johnson, Albrecht, Ketterings, Beckman, & Stockin, 2005). Crop residues have high carbon:nitrogen ratios which stimulate soil microbial activity, increase demand for nitrogen and lead to immobilization (Johnson, Albrecht, Ketterings, Beckman, & Stockin, 2005). Immobilization is the process where microorganisms uptake inorganic nitrogen compounds and make them unavailable to plants (Johnson, Albrecht, Ketterings, Beckman, & Stockin, 2005). Surface nitrogen application and several other factors potentially led to loss of applied nitrogen and crop residues at SAREC might have further promoted volatilization and immobilization of both applied and residual soil nitrogen. Thus, lower available nitrogen could be the reason for lower yield and quality at SAREC than at ShREC. Besides optimal soil pH for wheat is between 6.0-7.0 and a manganese deficiency may occur in soils above pH of 7 (Vitosh, 1998). The average pH of soil at ShREC (7.5) was lower than at SAREC (8.3) (Table 3.2). The higher pH and lower manganese at SAREC could also be a reason for the reduced performance at SAREC than ShREC. A combination of all these factors could be the reason for lower yield and protein at SAREC than at ShREC.

Moreover, the SAREC location had grasshopper herbivory on 30<sup>th</sup> July in 2019 which could potentially decrease the yield at SAREC. The damage occurred when spelt and emmer were at the milky ripe stage, wheat was in soft dough stage, and einkorn was at flowering. Effect of grasshopper damage on yield of wheat, emmer, and spelt at SAREC should be lower as they were already in late reproductive stages, but it may have contributed to the low yield of einkorn.

Both grain yield and grain protein was lower than at SAREC than at ShREC for all wheat species (Table 3.16) which suggests that SAREC may not be suitable to grow dryland modern and ancient spring wheat. Killen, Smith , Smith, Nelson, & Nachtman (2004) compared the spring wheat varieties growth in three University of Wyoming research stations and found that yield at the Powell was the highest followed by ShREC. However, the trial at Lingle, WY had an aphid infestation and there was not enough grain production to harvest. We could not find other studies on growing spring wheat in dryland fields at Goshen County, Wyoming (Lingle growing region) which suggests that spring wheat is not commonly grown in dryland fields here. Average dryland winter wheat yield in Lingle, WY was 3060 kg ha<sup>-1</sup> in 2016. Similarly, the average yield of spring wheat in dryland fields of Wyoming was 1143 kg ha<sup>-1</sup> in 2017 (USDA, 2019). Average spring wheat yield in our study was 875 kg ha<sup>-1</sup> at SAREC which is lower than the average yield of spring wheat in dryland fields of Wyoming in 2017.

The average yield of spring wheat cultivars in ShREC dryland in 2006, 2007, 2008, 2009, were 1385 kg ha<sup>-1</sup>, 2320 kg ha<sup>-1</sup>, 4896 kg ha<sup>-1</sup> and 2118 kg ha<sup>-1</sup> respectively (UW agricultural experiment station, 2019). The average yield of spring wheat in dryland fields of Wyoming was 1143 kg ha<sup>-1</sup> in 2017 (USDA, 2019). Average spring wheat yield at ShREC in our study was 2196 kg ha<sup>-1</sup> which is above the state average yield from 2017, and yields at University of Wyoming (UW) trial in 2006 and 2009 and close to the UW trial in 2007. The spring wheat in

our trial at ShREC obtained the expected yield and the yield of ancient wheat obtained in our study might be the standard yield in that growing location. Troccoli & Codianni (2005) reported the hulled yield of einkorn, emmer, and spelt as 3540 kg ha<sup>-1</sup>, 2800 kg ha<sup>-1</sup>, and 1420 kg ha<sup>-1</sup> respectively under rainfed condition in southern Italy. The hulled yield of einkorn, and emmer in our study (Table 3.16) is lower than their study. Castagna et al. (1996) reported that grain yield of einkorn, emmer, spelt, and modern wheat were 1060 kg ha<sup>-1</sup>, 2370 kg ha<sup>-1</sup>, 2710 kg ha<sup>-1</sup>, and 3860 kg ha<sup>-1</sup> and protein content ranged from 13-21%. Both protein content and yield of ancient wheats in their study was higher than in our study suggesting that ancient wheat species grown in Wyoming dryland may not be able to compete with ancient wheats grown in other places unless very high market price is provided. Though ShREC dryland had higher yield than the SAREC dryland, both sites had lower ancient wheat yields than the yields reported in other studies. Further studies on other growing regions of Wyoming as well as cost:benefit analysis of ancient wheats and other alternative crops is suggested to elucidate the full potential of growing ancient wheats in dryland, Wyoming.

stations, SAREC, Lingle, WY and ShREC, Sheridan, WY in spring 2019 and 2020.									
Crop	GDD till	DD till heading GDD 15 c		lays after	ays after GDD from			Whole season	
			head	heading		heading to harvest		GDD	
	SAREC	ShREC	SAREC	ShREC	SAREC	ShREC	S.	AREC	ShREC
				2019					
Einkorn	1424	1167	336	340	804	1058		2205	2065
Emmer	1111	949	335	324	835	951		1924	1878
Spelt	1267	1074	339	340	937	824		2182	1878
Wheat	973	818	334	317	904	922		1858	1861
				2020					
Einkorn	$\mathbf{NH}^{1}$	983	NH	309	NH	1074		NH	2036
Emmer	NH	902	NH	301	NH	874		NH	1758
Spelt	NH	902	NH	301	NH	1153		NH	2036
Wheat	NH	838	NH	272	NH	933		NH	1758

Table 3.23. Growing degree day (GDD) of wheat species grown at two University of Wyoming research

Table 3.23. Growing degree day in dryland sites.

<sup>1</sup>NH: No harvest

#### 3.4.3. Each ancient wheat species had unique growth, yield, quality, and WUE

Wheat species significantly affected growth, yield, quality and WUE in both locations (Table 3.10, 3.17 and 3.21). Plant stands of modern wheat was the highest, followed by einkorn, emmer and then spelt, similar to our irrigated studies (Table 3.11). And different hull and grain sizes might be the reason for population differences as each species has a different thousand seed weight (data not shown). Modern wheat headed 5-10 days earlier than emmer, 8-17 days earlier than spelt, and 12-27 days earlier than einkorn in our study (Table 3.11). Castagna et al. (1996) reported a similar finding in which modern bread wheat headed 10 days earlier than emmer, 16 days earlier than spelt and 24 days earlier than einkorn. This suggest that ancient wheats mature more slowly than modern wheat with einkorn being the slowest under Wyoming dryland conditions. Introducing ancient wheats in common crop rotation practice might require a change in crop rotation and crop management practices due to change in crop period. However, water used by all the wheat species was similar suggesting that they do not require a change in water management practice. Ancient wheats had higher NDVI than modern wheat at jointing and flag leaf stages suggesting that they had higher vegetative growth and potential to grow as forage crop than modern wheat (Table 2.14). Future research into biomass harvest could confirm the forage potential of these crops.

In ShREC, grain yield of modern wheat was 1.9 times that of emmer, 2.4 times of spelt yield, and 3.6 times of einkorn yield (Table 3.18). Grain protein of einkorn was the highest, but the difference in grain protein of einkorn and modern wheat (2.7%) was not as large as expected. Protein of emmer and spelt and modern wheat were all similar. Emmer could be the most suitable ancient wheat for Sheridan growing region if provided higher market price to balance lesser yield. With similar protein content as modern wheat, growing ancient wheats in Wyoming depends on the market price and consumer demand for them.

In SAREC, yield of modern wheat was 1.3 times of emmer yield, 2.4 times of spelt yield, and 5.6 times of einkorn yield (Table 3.18). Only spelt had higher protein than the modern wheat and the difference was not high (1.1%) (Table 3.18). Lesser yield of all the wheat species in SAREC compared to state average yield, suggest that there is low potential of introducing ancient spring wheats as alternative crop in Lingle growing regions. However, the result may vary by growing year and varieties used. Further studies in coming years using different varieties are suggested to evaluate the true yield potential. Previous studies found that ancient wheats were drought tolerant and suitable for marginal lands with low water inputs (Bencze et al., 2020; Konvalina et al., 2012). We found ancient wheats had similar water use as modern wheat and lower water use efficiency than modern wheat (Table 3.22). At ShREC, water use efficiency of einkorn emmer, and spelt were 6.8, 1.8, 2.1 times lower than modern wheat respectively. At SAREC, water use efficiency of einkorn emmer, and spelt were 5.2, 1.2, 2.4 times lower than modern wheat respectively. This suggests that ancient wheats require similar amount of water and can replace modern wheat in common crop rotation without alteration in water management, but they cannot perform better than modern wheat in low water input system and marginal conditions. Ancient wheats do not seem to be suited to low input marginal lands compared to modern wheat. However, the performance may vary by location, and varieties used. Future studies using multiple varieties of ancient wheats with addition of growing locations is suggested.

#### **3.5.** Conclusion

This study compared the performance of three different ancient wheat species (spelt, emmer, einkorn) and modern wheat under three different nitrogen treatments, two growing locations and dryland conditions. Pre-planting surface nitrogen application had no effect on growth, yield, and quality parameters of all the wheat species. No response to the applied nitrogen suggests that either nitrogen was lost, or low nitrogen is sufficient to obtain optimum yield in these locations. Future studies with other nitrogen rates and applying measures to reduce nitrogen loss such as soil nitrogen incorporation and split nitrogen application should be carried out to know the nitrogen demand of these ancient wheats under dryland conditions. Sheridan, WY was a more suitable location to grow both modern and ancient wheats than Lingle, WY under dryland conditions though the yield of ancient wheats was less than half the yield of modern wheat and the grain protein was only 0.4-2.7% higher than modern wheat. Among the ancient wheats, emmer, and spelt are suggested to be grown because of their higher yield compared to einkorn. Future studies in other locations in Wyoming are needed to identify the best ancient wheat production region. Future studies with split nitrogen application and multiple varieties of each crop should be conducted in several locations in WY. Research into market price and cost:benefit ratio to determine the actual production potential of these ancient wheats are also needed.

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# CHAPTER 4. CONCLUSION FOR GROWING ANCIENT WHEATS IN WY

In our study, ancient wheats, spelt, emmer, and einkorn were grown to identify their production potential in Wyoming. We evaluated the nitrogen demand, crop growth, and yield performance under multiple Wyoming growing conditions and locations. The study was conducted in three locations (Powell, Sheridan, and Lingle, WY), under dryland and irrigated conditions, and with three levels of nitrogen fertility applied.

There was no significant effect of pre-planting surface nitrogen application on the growth, yield, and quality of either ancient or modern wheats under irrigated or dryland conditions. Einkorn, emmer, and spelt had considerably lower yield, greater plant heights, increased lodging issues, slower maturation, and required an extra dehulling process compared to modern grains. Among the ancient wheats, emmer was the best suited in all locations and conditions with faster maturity, higher yield, and similar protein content as other ancient and modern wheat. In our study, the protein content of ancient wheats was slightly higher than modern wheat, but the difference was not significant in most of the cases. Replacing modern grains with ancient wheats will require price premiums and modification to standard small grain production practices to accommodate the differences in crop maturity, hulled nature, and lodging issues.

The performance of ancient wheats varied greatly by location with PREC, Powell, irrigated and ShREC, Sheridan, dryland being the most suitable locations compared to SAREC irrigated, Lingle, WY and SAREC dryland, Lingle, WY respectively. Einkorn grown in Powell under irrigated conditions had yield comparable to emmer and spelt but its yield was very low at the other study sites.

Future studies by adapting measures to reduce nitrogen loss such as soil nitrogen incorporation and split nitrogen application at various growth stages and including higher nitrogen rates will be needed to better elucidate the nitrogen demand of these ancient wheats. Other future studies with additional varieties, more locations, and economic analysis will be necessary to fully understand the true potential of ancient wheat in Wyoming.

# **APPENDICES**

# Appendix 1: Hail damage in irrigated study at SAREC in spring 2019

The effect of nitrogen treatment and wheat species (crop) on number of hail damaged heads m<sup>-1</sup> was tested after hail damage in irrigated field of SAREC on 9<sup>th</sup> July 2019. Nitrogen and the nitrogen by wheat species interaction had no effect on number of head damaged per meter row (Appendix a). Wheat species significantly affected number of head damaged per meter row. Emmer, modern wheat, and spelt had similar hail damage while einkorn had lower damage (Appendix b). Most of the einkorn plants had not headed out when hail damage occurred which was the reason for lower hail damage heads m<sup>-1</sup> of einkorn compared to other wheat species. Though einkorn had lower hail damage, it's yield was still lower than rest of the wheat species (Chapter 2, Table 2.17).

## Appendix a. Effect of Wheat Species and Nitrogen on Hail Damage

Appendix a. Analysis of variance showing P-Values for the effect of wheat species, nitrogen, and interaction between wheat species and nitrogen on number of head damaged per meter occurred on 9<sup>th</sup> July 2019 at the University of Wyoming James C. Hageman Sustainable Agriculture Research and Extension Center (SAREC), Lingle, WY.

Factor	Number of Heads Damaged m <sup>-1</sup>		
Crop	0.006		
Nitrogen	0.85		
Crop:nitrogen	0.78		

P-Value <0.05 are significant at 5% significance level.

#### Appendix b. Hail Damage Comparison by Wheat Species

Appendix b. Pairwise comparison for the effect of wheat species on hail damage occurred on 9<sup>th</sup> July, 2019at the University of Wyoming James C. Hageman Sustainable Agriculture Research and Extension Center (SAREC), Lingle, WY.

Crop	Number of Heads Damaged m <sup>-1</sup>	
Einkorn	7.9 B	
Emmer	21 A	
Spelt	13.6 AB	
Wheat	18.9 A	

Within columns, means followed by the same uppercase letters are not different at  $\alpha$ =0.05

#### Appendix 2. Grasshopper damage in dryland study at SAREC in spring 2019

The effect of nitrogen treatment and wheat species (crop) on percent of flag leaves damaged plot <sup>1</sup> and damage severity was tested. Ten random flag leaves plot<sup>-1</sup> were taken and assessed for feeding damage. For damage severity, each plot was evaluated by Horsfall-Barrat scale (Table 3.5; Francis, 2019). Nitrogen and the nitrogen by wheat species interaction had no effect on percent of flag leaves damaged plot<sup>-1</sup> or damage severity (Appendix c). Wheat species significantly affected percent of flag leaves damaged plot<sup>-1</sup> and damage severity (Appendix c). Emmer, spelt, and modern wheat had similar percent of flag damaged plot<sup>-1</sup> while einkorn had the lowest percent of flag leaves damaged plot<sup>-1</sup> (Appendix d). Damage severity of modern wheat was the highest, followed by emmer, then spelt, and then einkorn (Appendix d) All crops were in different growth stages when grasshoppers damaged the plot. Einkorn was in early vegetative growth stage (jointing) and shorter in height which might be reason for its lower damage compared to other crops. Modern wheat, emmer, and spelt were in late reproductive periods when grasshopper damage occurred. The effect of grasshopper infestation on yield and quality did not have a clear impact as the wheat species with higher grasshopper infestation had higher yield than the wheat species with lower infestation (Chapter 3, Table 3.17).

#### Appendix c. Effect of Wheat Species and Nitrogen on Grasshopper Damage

Appendix c. Analysis of variance showing P-Values for the effect of wheat species, nitrogen, and interaction between wheat species and nitrogen on percent of flag leaf damaged plot -1 and damage severity occurred on 30th July 2019 at the University of Wyoming James C. Hageman Sustainable Agriculture Research and Extension Center (SAREC), Lingle, WY under dryland condition.

Factor	Percent of flag leaves damaged plot <sup>-1</sup>	Damage severity
Crop	< 0.001	<0.001
Nitrogen	0.48	0.37
Crop:nitrogen	0.85	0.36

P-Value <0.05 are significant at 5% significance level.

### Appendix d. Grasshopper Damage Comparison by Wheat Species

Appendix d. Pairwise comparison for the effect of wheat species on percent of flag leaf damaged plot -1 and damage severity occurred on 30th July 2019 at the University of Wyoming James C. Hageman Sustainable Agriculture Research and Extension Center (SAREC), Lingle, WY under dryland condition.

Сгор	Percent of flag leaf damaged	Damage severity
	$plot^{-1}$	
Einkorn	20 B	13 C
Emmer	83 A	62 AB
Spelt	68 A	52 B
Wheat	70 A	71 A

Within columns, means followed by the same uppercase letters are not different at  $\alpha$ =0.05

#### Appendix 3. Nitrogen use efficiency (NUE) in irrigated fields

The effect of nitrogen treatment, and wheat species on NUE (Chapter 2, eqn 9) was tested.

Wheat species and nitrogen significantly affected NUE but the nitrogen by wheat species interaction was not significant (Appendix e). For all crops, nitrogen use efficiency decreased with increasing starting soil nitrogen except einkorn at SAREC whose NUE was very low and did not change with increasing starting soil nitrogen (Appendix f and g). This decrease in NUE corresponds to the lack of yield response to nitrogen (Table 2.14). At PREC, ancient wheats had similar nitrogen use efficiency, but their NUE was lower than barley (Appendix f). At SAREC,

nitrogen use efficiency of emmer and spelt was similar to modern wheat but NUE of einkorn was

lower than rest of the wheat species (Appendix g).

Appendix e. Effect of Wheat Species and Nitrogen on Irrigated Nitrogen Use Efficiency

Appendix e. Analysis of variance showing P-Values for the effect of wheat species, nitrogen, and interaction between wheat species and nitrogen on nitrogen use efficiency at the irrigated fields of SAREC, Lingle, WY, and PREC, Powell, WY, in spring 2019 and 2020.

Parameter	Factor	SAREC	PREC
Nitrogen Use	Crop	0.03	< 0.001
Efficiency	Nitrogen	0.01	0.02
	Crop:nitrogen	0.56	0.6

P-Value <0.05 are significant at 5% significance level.



Appendix f. Nitrogen Use Efficiency at PREC Irrigated

Appendix f. Nitrogen use efficiency of spring ancient wheats and barley with increasing starting soil nitrogen at irrigated fields of PREC, Powell, WY in 2019, and 2020. Starting soil nitrogen is the sum of residual soil nitrogen and applied soil nitrogen.



Appendix g. Nitrogen Use Efficiency at SAREC Irrigated

Appendix g. Nitrogen use efficiency of spring ancient wheats and modern wheat on increasing starting soil nitrogen at irrigated fields of SAREC, Lingle, WY in 2019 and 2020. Starting soil nitrogen is the sum of residual soil nitrogen and applied soil nitrogen.

# Appendix 4. Nitrogen use efficiency in dryland fields

The effect of nitrogen treatment and wheat species on NUE was tested in dryland fields of SAREC and ShREC. Wheat species, and nitrogen significantly affected NUE in both sites (Appendix h). Nitrogen by wheat species interaction had significant effect on NUE at SAREC but not ShREC (Appendix h). For all crops, nitrogen use efficiency decreased with increasing starting soil nitrogen (Appendix i and j) corresponds to the lack of yield response to nitrogen. Nitrogen use efficiency of modern wheat was the highest, followed by emmer, spelt, and then einkorn specially at higher starting soil nitrogen in both sites (Appendix i and j). Nitrogen use efficiency at lower nitrogen seems be similar among modern wheat, emmer, and spelt at low nitrogen in SAREC (Appendix i).

# Appendix h. Effect of Nitrogen and Crop on Nitrogen Use Efficiency

Appendix h. Analysis of variance showing P-Values for the effect of wheat species, nitrogen, and wheat species by nitrogen interaction on nitrogen use efficiency at the dryland fields of James C. Hageman Sustainable Agriculture Research and Extension Center (SAREC), Lingle, WY, and Sheridan Research and Extension Center, Sheridan, WY in spring 2019 and 2020.

Parameter	Factor	SAREC	ShREC
Nitrogen Use	Crop	< 0.001	< 0.001
Efficiency	Nitrogen	< 0.001	< 0.001
	Crop:nitrogen	0.01	0.19

P-Value <0.05 are significant at 5% significance level. SAREC field was lost in 2020 due to grazing damage.



Appendix i. Nitrogen Use Efficiency at SAREC Dryland

Appendix i. Nitrogen use efficiency of spring ancient wheats and modern wheat on increasing starting soil nitrogen at dryland fields of SAREC, Lingle, WY in 2019. Starting soil nitrogen is the sum of residual soil nitrogen and applied soil nitrogen.



Appendix j. Nitrogen Use Efficiency at ShREC Dryland

Appendix 4.c. Nitrogen use efficiency of spring ancient wheats and modern wheat on increasing starting soil nitrogen at dryland fields of ShREC, Sheridan, WY in 2019 and 2020. Starting soil nitrogen is the sum of residual soil nitrogen and applied soil nitrogen.