Characterization of crested wheatgrass germplasms for plant maturity and associated physiological and morphological traits

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Samuel Tandoh

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ABSTRACT

Crested wheatgrass [*Agropyron cristatum* (L.) Gaertn.] is a drought tolerant, winter hardy perennial grass used for early spring grazing in western Canada. This grass matures early, and mature plants are not palatable for grazing animals. The objectives of this study were: 1) determine DNA content and ploidy level of 45 crested wheatgrass accessions 2) to characterize crested wheatgrass germplasm for plant maturity and associated agronomic characteristics to identify superior germplasm with late maturity; 3) to evaluate flowering time of selected germplasms of crested wheatgrass under a controlled environment. A field plot was established using 45 crested wheatgrass accessions in July 2014 at Agriculture and Agri-Food Canada (AAFC) Saskatoon Research Center at Saskatoon SK, Canada using a randomized complete block design with four replications with data collected in 2015, 2016 and 2017. On the basis of DNA content (pg $2C^{-1}$ = DNA content of diploid somatic nucleus), mean DNA content was 14.12 pg $2C^{-1}$ for diploid, 28.02 pg $2C^{-1}$ and 39.48 pg $2C^{-1}$ for tetraploid and hexaploid crested wheatgrass, respectively. Among the 45 accessions, there were 8 diploid, 31 tetraploid, and 6 hexaploid accessions. Plant maturity and other measured characteristics differed significantly among the ploidy levels. Days to heading, plant height, leaf-to-stem ratio, forage DM yield, leafiness and plant vigor and nutritive value (crude protein, neutral detergent and acid detergent fibers) differed significantly ($P \leq 0.05$) among accessions at flowering stage. In this study, days to heading showed a positive correlation with leaf-to-stem ratio ($r=0.23$, $P<0.0001$), indicating that selection for later maturity in crested wheatgrass may lead to an increase in leafiness. When all 45 accessions were considered, there was a non-significant correlation between days to heading and DM yield ($r= 0.07$, $P=0.09$), but this relationship was significant ($r=0.34$, $P<0.0001$) when only Canadian breeding lines and cultivars were considered. Based on agronomic performance and nutritive value, the 45 crested wheatgrass accessions were grouped into three main clusters. In addition, ranking of days to heading among selected accessions was consistent in field and controlled environments. In conclusion, plant maturity varied within- and among- accessions, among ploidy levels, and selection for late maturity may simultaneously increase forage DM yield and leaf-to-stem ratio in crested wheatgrass. Information obtained from this study on agro-morphological traits, nutritive values and ploidy determination among the 45 crested wheatgrass accessions will be useful for future crested wheatgrass breeding programs.

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DEDICATION

I dedicate my dissertation work to my lovely wife Joycelene, my parents, Thomas and Dorothy Tandoh and my brothers, Solomon and Frank as well as my sisters Esther and Gladys whose words of encouragement and push for tenacity ring in my ears.

I dedicate this work and give special thanks to my brother in -law Kwaku Boakye for being there for me throughout the entire master’s program.

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LIST OF ABBREVIATIONS

Abbreviation	
AAFC	Agriculture and Agric-Food Canada
ADF	Acid Detergent Fiber
AFLP	Amplified Fragment Length Polymorphisms
ANOVA	Analysis of Variance
CO	CONSTANS
CP	Crude Protein
DAPI	4’6-diamidino-2-phenylindole
DM	Dry Matter
DNA	Deoxyribonucleic acid
FCM	Flow cytometry
FLC	FLOWERING LOCUS C
FRI	FRIGIDA
FT	FLOWERING LOCUS T
GEBV	Genomic Estimated Breeding Value
IVDDM	In Vitro Digestible Dry Matter
LD	Long Daylength
LSD	Least Significant Difference
NDF	Neutral Detergent Fiber
NPGS	National Plant Germplasm Systems
PCA	Principal Component Analysis
PIF4	Phytochrome Interacting Factor 4
RCBD	Randomized Complete Block Design
RFLP	Restriction Fragment Length Polymorphisms
RNA	Ribonucleic acid
RRPS	Restricted Recurrent Phenotypic Selection
SNP	Single Nucleotide Polymorphism
SSR	Single Sequence Repeat
UPGMA	Unweighted Pair-group Method with Arithmetic mean
USDA	United States Department of Agriculture
VRN1	VERNALIZATION 1
VRN2	VERNALIZATION 2
VRN3	VERNALIZATION 3

1. 0 Introduction

The beef and dairy industries are important to the Canadian economy with both industries contributing more than \$11 billion directly, and \$50 billion indirectly (Yungblut 2012). Forage crops provide about 80% of beef animal's diet, whether it is providing summer pasture or winter feed (Canfax Research Services 2014). Approximately 40% of Canadian farmland is used for grazing and growing forage crops (Yungblut 2012). With the beef industry being one of the major sectors of agriculture with close to 2.3 million cattle and calves in Saskatchewan (Canfax Research Services 2016), the need for forage crops with characteristics of high yield, high quality and good adaption has increased to reduce feed cost of the ever-increasing beef industry in Saskatchewan and in Canada (BCRC, 2016).

Crested wheatgrass (*Agropyron cristatum* L.), a member of *Poaceae* family, is a long-lived, cool-season grass with extensive root systems (Iwaasa et. al 2014; Yu et al. 2012). It is tolerant to drought and extreme winter temperatures and has wide adaptability to abiotic stresses (Asay and Jensen 1996**,** Yu et al. 2014). It is one of the first perennial grasses to green up in the spring and it is of high forage quality at the early growth stages (Hoffman et al. 1993). Therefore, crested wheatgrass is widely used for early spring grazing in the Brown and Dark Brown soil zones of Saskatchewan. The early grazing of this species shortens the winter-feeding period and provides forage until the later developing native range grasses are ready to graze in early summer (Dwyer and Owen 1984). Since its introduction in North America, crested wheatgrass has provided an excellent source of forage to cattle, sheep, horse and other livestock due to its nutritional properties, and palatability (Ray et al. 1997; Asay et al. 2003; Li et al. 2004). Previous studies have shown that steer weight gains on crested wheatgrass pasture ranged from 0.82 to 1.59 kg day^{-1} (Hart et al. 1983; Hofmann et al. 1993; Karn et al. 1999). Livestock weight gains from crested wheatgrass decrease as the plant matures, as it becomes coarse and unpalatable (Hart et al. 1983, Larson et al. 2018). Daugherty et al. (1982) reported that mature crested wheatgrass plants not only have low leaf-to-stem ratio, and contain high fiber concentrations. When mature plants remain ungrazed in a given year, standing dead stems often persist, which can result in substantial loss of pasture forage and animal production if occurring in high densities (Iwaasa et al. 2014). This calls for the development of later maturing crested wheatgrass cultivars with a better potential for pasture utilization. However, genetic variation of plant maturity in crested wheatgrass is not well documented. There is a need to evaluate and characterize a diverse collection of crested wheatgrass germplasm for flowering time, plant maturity and associated forage traits to help extend the grazing window of crested wheatgrass. It was hypothesized that: 1) crested wheatgrass accessions differ in ploidy level, which would cause variation in plant maturity and other agronomic characteristics; 2) agronomic and morphological traits will vary among different crested wheatgrass germplasm and cultivars; 3) genetic variation exists within and among different crested wheatgrass cultivars and germplasm for plant maturity and other agronomic characteristics; 4) plant maturity and phenotypic characteristics of selected germplasms of crested wheatgrass under a controlled environment will be consistent with field environment. The objectives of the present study were: 1) to determine ploidy level of crested wheatgrass germplasm collections; 2) to determine the agronomic and morphological characteristics and nutritive value of crested wheatgrass cultivars and new germplasm introductions from world gene banks in the field; 3) to evaluate flowering time of selected germplasms of crested wheatgrass under a controlled environment.

2. 0 Literature Review

2.1 Description and adaptation

2.1.1 Wheatgrass species

There are 100-150 wheatgrass species, of which 22 to 30 of are considered to be native to the North America and about 100 are introduced from Eurasia (Rogler 1973; Cronquist et al. 1977). The important wheatgrasses are perennial, cool-season species, including native and introduced grasses (McDonald and Copeland 2012)**.** The introduced wheatgrass such as crested wheatgrass, intermediate wheatgrass [*Thinopyrum intermedium* (Host) Z.-W. Liu & R.-C. Wang], and tall wheatgrass [*Thinopyrum ponticum* (Podp.) Barkworth & D.R. Dewey] are important forage grasses, and widely seeded in the Canadian prairies. Native wheatgrass such as western (*Pascopyrum smithii*), northern wheatgrass (*Elumus lanceolatus*), and slender wheatgrass (Elymus trachcaulus) are also dominant in many prairie areas in the Central and Northern Great Plains, the Intermountain region, and high altitudes in the Rocky Mountains of the U.S and Canada (Hitchcock 1951). Most of wheatgrasses are adapted to areas of steppe or desert with sub-humid to arid climatic conditions. The wheatgrass inflorescence is a terminal spike with multi-flowered spikelets (Asay and Knowles 1985), which is similar to annual cereal wheat (*Titricum aestivum*). Asay and Knowles (1985) reported that both caespitose (bunch) and rhizomatous (sod-forming) types are common.

Hitchcock (1951) and Bowden (1965) included wheatgrasses in the genus *Agropyron* even though the taxonomy of this group of grasses has been in an unsettled state for several years. Asay and Knowles (1985) reported that wheat (*Triticum* spp.), barley (*Hordeum* spp.), rye (*Secale cereal* L.), and other genera of forage grasses including *Elymus* and *Sitanion* were all related to wheatgrass species. Recent revisions in taxonomy of *Agropyron* have reduced the species in the crested wheatgrass complex based on genomic and morphological relationships (Dewey, 1969; Yen et al. 2005). This complex includes the diploid form 'Fairway' type – [*A. cristatum* (L.) Beauv. ssp. *Pectinatum* (Bieb.) Tzvel.]; 'Standard' type, [*A. desertorum* (Fisch. Ex Link) Schult]; and 'Siberian' type, [*A. fragile* (Roth) Candargy var. *sibiricum* (Willd.) Tzvel.] (Asay and Knowles 1985). Self-fertile and caespitose species such as slender wheatgrass [*E. trachcaulus*] and bearded wheatgrass, previously known as *A. trachycaulum* (Link) Malte and *A. subsecundum* (Link) Hitchc. respectively, are included in the genus *Elymus* (Asay and Knowles 1985). Thickspike wheatgrass [*A. dasystachynum* (Hook.) Scribn.] is classified as *E. lanceolatus* (Scribn. & Smith) Gould, and western wheatgrass [*A. smithii* (Rydb.)] is now described as *Pascopyrum smithii* (Rydb.) Love (Asay and Knowles 1985). Intermediate wheatgrass [*Elytrigia intermedia* (Host) Nevski] and tall wheatgrass [*Et. pontica* (Podp) Holup] which were previous classified under *Agropyron* have all been classified under genus *Thinopyrum.* Bluebunch wheatgrass [*Pseudoroegneria spicata* (Pursh) A. Love] formerly *Agropyron spicatum* has also been classified under the genus *Pseudoroegneria*. Asay and Knowles (1985) indicated that the taxonomic alignment is compatible with the biological relationships and the philosophy of Eurasian botanists working in the native habitat of most wheatgrass species.

2.1.2 Crested wheatgrass

The inflorescence of crested wheatgrass is flat, tapering towards the tip. Spikes are from 3.8 to 7.6 cm long (Ogle 2006). Asay and Knowles (1985) reported that the shape of the spike is an important character in the classifying crested wheatgrass. The spikelets are overlapping and placed flat on the rachis, containing 4 to 8 overlapping florets per spikelet. Individual seed is covered by palea and lemma that are generally tapering to the tip or with short awns. Stems are 60 to 90 cm in height and grow in dense bunches. Leaves are profuse at the base and along the stem, and pubescent on the upper surface. Roots are fibrous, finely branched and extend deep into the soil to a depth of about 2 m.

Crested wheatgrass occurs in diploid (2n=2x=14), tetraploid (2n=2x=28) and hexaploid (2n=2x= 42) forms (Copete et al. 2018). Fairway crested wheatgrass is a diploid form with spikes towering 2 to 7 cm long. The spikelets are more widely spreading with the glumes somewhat contoured, gradually tapering into awn of 0.2 to 0.5 cm long. It is shorter and leafier than the standard type and has short-broad spikes with smaller seeds (Ogle 2006). The standard type is a tetraploid (2n=4x=28) with varying spike shape from comb-like to oblong. Spikelets are flattened, closely overlapping, oriented at a slight angle on the rachis. Lemmas are linear-lanceolate narrowing to a short awn. Glumes are awl shaped, firm, and keeled. Leaves are flat, smooth below, slightly scabrous (coarse) above and vary in width from 0.2 to 0.6 cm. Siberian wheatgrass is similar to fairway and standard crested wheatgrass except it has finer leaves and stems, narrower and awnless glumes and lemmas and spikelet are more ascending. Siberian wheatgrass is more drought tolerant and retains its greenness and palatability later into the summer than either standard or fairway wheatgrass (Ogle 2006).

2.2 Forage characteristics of crested wheatgrass

2.2.1 Establishment and adaptation

Crested wheatgrass has been selected for forage productivity, seed production, ease of establishment, and adaptation. Crested wheatgrass has a high germination rate for a wide range of conditions and establishes well under less than ideal conditions (Holechek 1981). Even though companion crop provides some revenue when grown together during the establishment year, the seed production of crested wheatgrass has shown to be higher when no companion crop is sown with it (Buglass 1964; Elliot 1973).

Crested wheatgrass is competitive when established and can maintain productivity for more than 30 years (Hardy 1989). It is also known to be resistant to harsh weather conditions such as drought and extreme cold temperature (Shiflet 1994). Its upright growth habit and high forage yield potential makes it important hay grass in the drier region of western Canada. The drought tolerance of crested wheatgrass has been demonstrated by excellent growth and survival of this species during the drought in the 1930's in the United States of America and Canada (Hubbard 1949). Its deep root and tendency to go dormant in dry years contribute to its drought tolerance, especially for the Standard type, which is more drought tolerant than the Fairway type (Asay 1992). Busso et al. (1990) also noticed that crested wheatgrass had the potential to recover rapidly after a drought due to high accumulation of total non-structural carbohydrate reserves in the lower stem bases and root system.

2.2.2 Forage nutritive value

It's considered a desirable forage for livestock and wildlife because of its ability to consistently produce high quality forage in the early spring and fall. In addition, it can withstand heavy grazing in the spring (Pavlychenko 1942; Hydes and Sneva 1963; Frischknecht and Harris 1968). Grasses are one of the primary feed sources for ruminant feed and generally grow in marginal soils under nitrogen-limiting conditions (Wilkins and Humphreys 2003). Livestock performance is the ultimate determination of forage quality, but estimates of forage quality constituents, including crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), and in vitro digestible dry matter (IVDDM) can be used to predict animal performance.

Crude protein is the amount of nitrogen from both protein and non-protein sources in the forage and is predictive of available protein. Acid detergent fiber measures cellulose and lignin in cell wall portions of the forage and is used as an indicator for forage digestibility with ADF negatively correlated to digestibility. Neutral detergent fiber measures the total cell wall content of the forage including hemicelluloses and NDF is a negative indicator of animal dry matter intake. In-vitro digestible dry matter estimates the amount forage that is potentially digestible by the ruminant in the rumen. The forage nutritive value of crested wheatgrass varied among growth stages, growing environment conditions, and soil nutrients (Table 2.1, 2.2 and 2.3).

Table 2.1 Crude protein (CP) concentration (% DM) of crested wheatgrass at different growth stages

Growth stage	CP	Reference
Vegetative	14.4-22.8	Hathway and Pirelli 2004; Whitman et al. 1951; Coulman (unpublished data)
Boot	12.2-19.3(2-yr mean)	Coulman (unpublished data)
Heading	11.9-12.4(2-yr mean)	Coulman (unpublished data)
Anthesis	10.3-11.4(2-yr mean)	Coulman (unpublished data)
Seed development	6.5-10.2	Glover et al. 2004; Whitman et al. 1951
Seed maturity	3.6-9.0 (over a 2-yr period)	Biligetu et al.2014; Glover et al. 2004

Crude protein concentration (%DM) ranged from 14.4–22.8 at vegetative stage, 12.2-19.3 at boot stage, 11.9-12.4 at heading stage, 10.3-11.4 at anthesis , 6.5-10.2 5 at seed development stage, and 3.9-9.0 at seed maturity stage (Table 2.1).

Table 2.2 Neutral detergent fiber (NDF) concentration (%) DM of crested wheatgrass at different growth stages

Growth stage	NDF	Reference
Vegetative	42.2-53.9(2-yr mean)	Coulman (unpublished data)
Booting	51.1-61.1(2-yr mean)	Coulman (unpublished data)
Full heading	58.8-60.2(2-yr mean)	Coulman (unpublished data)
Anthesis	60.0-61.2(2-yr mean)	Coulman (unpublished data)
Seed development	55.2-61.8 (over a 2 yr period)	Glover et al. 2004
Seed maturity	60.2	Biligetu et al.2014

Concentrations of NDF (%DM) were 42.2–53.9 at vegetative stage, 51.3–61.1 at boot stage, 58.8–60.2 at full heading stage, 60.0–61.2 at anthesis stage, 55.2–61.8 at seed development stage, and 60.2 at seed maturity stage (Table 2.2).

Table 2.3 Acid detergent fiber (ADF) concentration (% DM) of crested wheatgrass at different growth stages

Growth stage	ADF (%DM)	Reference
Vegetative	19.5-32.0(2-yr mean)	Coulman (unpublished data)
Booting	24.6-35.8(2-yr mean)	Coulman (unpublished data)
Full heading	33.0-33.4(2-yr mean)	Coulman (unpublished data)
Anthesis	34.7-36.2(2-yr mean)	Coulman (unpublished data)
Seed development	30.3-34.6 (over a 2 yr period)	Glover et al. 2004
Seed maturity	34.2	Biligetu et al.2014

Concentrations of ADF (%DM) were 19.5–32.0 at vegetative stage, 24.6–35.8 at boot stage, 33.0–33.4 at full heading stage, 34.7–36.2 at anthesis, 30.3–34.6 %DM at seed development stage and 34.2 at seed maturity stage (Table 2.3).

2.2.3 Forage dry matter (DM) yield

Improvement of forage DM yield has been the primary aim of many forage breeding programs, and genetic gain have been realized in most species over the years (Vogel et al. 1989; Barnes et al. 2003; Wilkins and Humphreys 2003). Diploid (Fairway type) crested wheatgrass yields approximately 3772 kg ha^{-1} in the Brown soil zone, 5090 kg ha^{-1} in Dark Brown soil zone, 4863 kgha^{-1} in the Black and Grey soil zones while tetraploid crested wheatgrass cultivars, ‘Nordan’ and ‘Summit’, yield approximately 3590 kg ha^{-1} in the Brown soil zone, 5363 kg ha^{-1} in the Dark Brown soil zone, 5227 kgha^{-1} in the Black and Grey soil zones in Saskatchewan, Canada (Sask. Forage Council 2007).

Table 2.4 Forage dry matter yield (Kg ha^{-1}) of crested wheatgrass with different ploidy forms.

Cultivar	Ploidy	Yield	Reference
AC Goliath	Tetraploid	7570- 8970 kg ha^{-1} (2-yr mean)	Jefferson and Coulman (2008)
Kirk	Tetraploid	3380 kg ha^{-1} (6-yr mean)	Jefferson and Cutforth (2005)
Kirk	Tetraploid	7630-9770 kg ha^{-1}	Jefferson and Coulman (2008)
Nordan	Tetraploid	7078-7482 kg ha^{-1}	Baenziger and Knowles (1969)
Parkway	Diploid	3500 kg ha^{-1} (6-yr mean)	Jefferson and Cutforth (2005)
Parkway	Diploid	6460-7870 kg ha^{-1}	Jefferson and Coulman (2008)
Summit	Tetraploid	7440-10186 kg ha^{-1} @ 400 N kg ha^{-1}	Lawrence and Knipfel (1981)
Summit	Tetraploid	6093-10167 kg ha^{-1} @ 200 N kg ha^{-1}	Lawrence and Knipfel (1981)
Summit	Tetraploid	4747-8018 kg ha^{-1} with no N fertilization	Lawrence and Knipfel (1981)

In various studies, forage DM yield of crested wheatgrass ranged from 1278-10,186 kg ha^{-1} depending on growth environment, N fertilizer application, and cultivar (Table 2.4). Forage DM yield was 3920-4975 kg ha^{-1} in the earlier report of Baenziger and Knowles (1969), but it has dramatically increased in more recent studies of Jefferson and Cutforth (2005) and Jefferson and Coulman (2008). This yield increase may be because of continuous breeding effort of this species in western Canada.

2.2.4 Seed yield

Marshall and Wilkins (2003) indicated that seed yield plays an important role in the commercial success of forage grass cultivars. Seed yield has been known to be a complex trait which is affected by several yield components and the environment (Boelt and Studer 2010). In tall fescue, seed yield was found to be positively related to dry matter yield and plant height (Veronesi and Falcinelli 1988). Langer (1980) reported that the number of spikes per unit area affect seed yield in ryegrass and cereals. However, seed yield was found not to be related to plant height in switchgrass (Newell and Eberhart 1961) and not related to dry matter yield in sainfoin

(Bhattarai et al. 2018). The average seed yields of crested wheatgrass are 371 kg ha^{-1}, but yield as high as 1112 kg ha^{-1} have been reported in Saskatchewan (Anonymous 1998). Seed yield of crested wheatgrass is influenced by several agronomic practices such as row spacing, and fertilization including type, rate, timing, and method of application (Buglass 1964; Knowles 1956; Knowles and Buglass 1966; Rogler 1960). A number of studies have been conducted to understand the effect of row spacing on seed yield of crested wheatgrass. Canode (1968) found that ‘Nordan’ crested wheatgrass had about 75 kg ha^{-1} higher seed yield when grown at a row spacing of 60 cm as compared to 30 or 90 cm at Pullman, Washington in United States of America. The seed yields of ‘Nordan’ crested wheatgrass averaged across five seed production years were found to be higher when the crop was sown at a row spacing of 61 cm than it was at 15, 30, or 46 cm (McGinnies 1971). Based on a five-year study of ‘Fairway’ and ‘Summit’ crested wheatgrass sown with row spacing of 30 and 91 cm at Scott, Saskatchewan, Canada, Crowle (1966) reported that average seed yields for the wider row spacing (91 cm) was 160-220 kg ha^{-1} more on both dryland and irrigated trials. Darwent et al. (1987) found that seed yield increased as the row spacing widened from 16 cm up to 104 cm over a three-year period.

Similar to other grasses, the application of N fertilizer is generally considered as an important factor in crested wheatgrass seed production (Fulkerson et al.1951; Knowles and Cooke 1952; Harlan and Kneebone 1953; Stitt et al. 1954; Buller et al. 1955). Crowle (1966) reported an increase of seed yields by 250 and180 kg ha^{-1} for ‘Fairway’ and ‘Summit’, respectively, after broadcasting 56 kg N ha^{-1} during later September.

Seed yield of crested wheatgrass is high when no companion crop is sown because of rapid seedling establishment. Although the companion crop provides some revenue during the establishment year, the yield of first seed crop is sufficiently reduced to offset the benefit of the companion crop. The first-year seed yield of 'Summit' crested wheatgrass was increased from 55 kg ha^{-1} with wheat as companion crop to 215 kg ha^{-1} without a companion crop (Buglass 1964). Over six-years of harvest, Elliot (1973) reported that seed yield of 'Fairway' crested wheatgrass ranged between 85 and 210 kg ha^{-1} year when no companion crop was sown. Lawrence (1970) compared seven row spacing for wheat under sown with 'Summit' crested wheatgrass. The number of established seedlings per meter of row was not significantly different among the treatments. The mean seed yield of 'AC Goliath' was higher (417 kg ha^{-1}) than Kirk (303 kg ha^{-1}) and Fairway (224 kg ha^{-1}) under 30 cm row spacing over a 3-yr period at Saskatoon (Coulman 2006).

2.3 Forage breeding and genetics

Cultivated crops have been selected for desirable traits for thousands of years by humans (Mellish 2001). Vogel and Sleper (1994) reported that breeding efforts in forages started formally in the early stages in the late 19th century with serious improvements made in the 1950's. *Agropyron* species which are members of the perennial *Triticeae*, has been one of the forages grasses that have received the most attention from plant breeders (Asay 1992). Traits such as forage yield, seed yield, forage quality (digestibility, grass tetany potential, and protein concentration), and stand establishment have been the most prominent traits that have been selected for in crested wheatgrass populations (Mellish 2001). Polyploidy is also commonly found in many forage species complicating the inheritance of traits, but Nguyen and Sleper (1983) indicated that breeding should been focused on the production of improved heterogenous populations in these species. Most perennial forages grasses such as crested wheatgrass are self-incompatible, outcrossing perennial grasses. Vogel and Lamb (2007) described the breeding system in crested wheatgrass as "a breeding system which involves population genetics and uses recurrent selection or repeated generations of breeding". The goal of outcrossing breeding is to change population means for specific traits by increasing the frequency of desirable genes with improved populations released as synthetic cultivars (Vogel and Lamb 2007). In such a breeding system, it is important to identify and suppress environmental factors might have on a particular phenotype to estimate the true genetic differences within and among genotypes and families.

The most common breeding method in perennial forage breeding is recurrent selection with or without progeny testing (Conaghan and Casler 2011). Within the recurrent selection, Vogel and Pedersen (1993) reported that restricted recurrent phenotypic selection (RRPS) and family selection are two popular recurrent selection systems. In RRPS, a large number of individual genotypes are evaluated for a few years for a trait or traits of interest, followed by identification, selection and isolation of best individuals and intercrossing of the best individuals. Family selection is a type of breeding system which uses family information to select superior individuals. It improvement on the basis of individual selection becomes difficult because of environmental variation, and family selection method is useful. Family selection is also sub-grouped into between or within family selection. It might require about 10-15 years for the development and registration of a new forage cultivar (Posselt 2010). Conventional breeding method in perennial grasses is generally dependent on phenotypic selection and improvement of their adaptation requires a considerable amount of efforts and several evaluation cycles under multiple growth environments (Vogel and Pedersen 1993).

The end result of most forage breeding programs is the production of a synthetic cultivar or open pollinated cultivars (Poehlman and Sleper 1995). Both populations are similar in that they are derived from an open pollination of selected clones, stains, or inbred lines. However, they are different in the sense that, a synthetic cultivar must be occasionally reconstituted by inter-pollinating components to produce additional basic seed while open pollinated cultivars are not reconstituted from the original component. In forage breeding, synthetic cultivars are popular as they take advantage of the self-incompatible, outcrossing nature of most forage species, and produce an adequate amount of seeds in early generations (Poehlman and Sleper 1995). Knowles (1959) explained that parents for synthetic cultivars are chosen based on their general combining ability (GCA) which is determined by a progeny test. The aim of a synthetic cultivar is to maximize the combination of favorable alleles while reducing the effect of inbreeding depression (Casler et al. 1996). This is accomplished by choosing an optimum number of plants to form the synthetic. A smaller number of superior combining clones results in higher yield in the first synthetic generation but increases the potential of inbreeding depression in further generations.

Genomic selection is a breeding method that uses genomic estimated breeding value (GEBV) to predict genetic value of selected candidates (Lorenz et al. 2011). In recent years, marker assisted breeding and genomic selection have become important breeding techniques in many forage crops such as alfalfa and switchgrass (*Panicum virgatum* L.), perennial ryegrass (*Lolium perenne* L.), white clover (*Trifolium repens* L.) (Hayward et al. 1994; Casler 2012; Ravagnani et al. 2012; Lipka et al. 2014; Annicchiarico et al. 2015; Li et al. 2015; Guillaume et al. 2016; Biazzi et al. 2017). To date, no report is available on genomic selection in crested wheatgrass, but different molecular markers were applied to understand genetics of crested wheatgrass. Che et al. (2008) characterized selected crested wheatgrass lines using Simple Sequence Repeats (SSR) marker. Mellish et al. (2002) studied the inter-population relationship and genetic diversity within and among populations from *Agropyron* species using Amplified Fragment Length Polymorphisms (AFLP), where the majority of the variability was found within populations. Substantial genetic diversity was found among different *Agropyron* species using the protein marker gladin (Chen and Li 2007; Miao et al. 2011; Chen et al. 2013). The relationship, diversity and ploidy level of species in the genus *Agropyron* were studied using morphological, taxonomic and cytological information (Dewey and Pendse 1967; Dewey and Asay 1982).

2.3.1 Crested wheatgrass introduction and breeding in Canada

Smoliak and Bjorge (1981) reported that crested wheatgrass was first introduced to the University of Saskatchewan in 1911 and distributed throughout western Canada in 1927. In 1922, the first crested wheatgrass breeding program was initiated at the University of Saskatchewan. The first improved cultivar was the diploid cultivar ‘Fairway’, which was released by L. E. Kirk at Saskatoon in 1932 (Elliot and Bolton 1970). Since then, many diploid and tetraploid cultivars have been released. Hein (1955) reported that Standard type crested wheatgrass ‘Nordan ‘was released in 1953. ‘Parkway’ (1969) and ‘Parkland’ (1999), two important diploid cultivars, were developed and released by Canada Department of Agriculture Research Station at Saskatoon, Saskatchewan. ‘Parkway’ produces good seeds and has a good lodging resistance (Ogle 2006). In recent years, tetraploid cultivars have been developed, which include ‘Kirk’ (1987), ‘AC Goliath’ (2006), and ‘New Kirk’ (2012). Plant height of ‘AC Goliath’ ranges from 63 to 104 cm (Mellish and Coulman 2002). With respect to tiller density and weight, ‘AC Goliath’ produced fewer tillers, but the average weight of each tiller was greater than other crested wheatgrass cultivars tested (Coulman 2006). Cultivar ‘Kirk’ is characterized by the tall growth habit of ‘Nordan’ and the wide spiked habit of ‘Fairway’. It is slightly greyish green at flowering as compared to ‘Nordan’ and ‘Fairway’, which exhibits bright green. ‘Kirk’ is recommended for hay and early spring grazing under moderately moist conditions (Knowles 1990). Cultivar ‘NewKirk’ is well adapted to both hay and pasture production in all regions of western Canada, it is slightly taller and has longer, wider leaves and crown than Kirk (SeCan 2015). A hexaploid cultivar, ‘Douglas’, was released in 1994 by USDA-Utah Range and Forage Research Center. ‘Douglas’ is characterized by larger seeds as compared to other crested wheatgrasses and has excellent seedling vigor. It produces less forage dry matter yield than the other cultivars at Saskatoon (Ogle 2006). With respect to its leafiness, Ogle (2006) reported that it is leafier, and its leaves remain green into the growing season. It has a better forage preference rating and performs better under 330-360 mm or higher annual precipitation (Ogle 2006).

2.3.2 Breeding for late maturity

A major challenge that has arisen with respect to producing high quality forage from crested wheatgrass is its short growth window, and in many cases, forage nutritive value declines rapidly after plant head emergence. Casler et al. (1996) reported that heading date, anthesis date, or a relative maturity score on a calendar date is the most commonly applied selection criterion in breeding cool-season forage grasses for late maturity. The variation in relative maturity of reproductive growth in many cool-season grasses has been exploited in developing optimum management strategies (Casler et al. 1996). In orchardgrass (*Dactylis glomerata* L.), for example, late-maturing cultivars were reported to be less persistent in mixtures with alfalfa than early maturing cultivars (Casler 1988; Casler and Walgenbach 1990). Humphreys and Eagles (1988) noticed late-heading lines of perennial ryegrass were more tolerant to freezing temperature than the early heading lines. Casler (1990) indicated that, late-maturity cultivars yielded higher forage nutritive value at the reproductive growth stage and sometimes even at the vegetative growth stage. Plant maturity, despite being highly heritable and under the control of many genes, can be readily amended to selection (Cooper 1959; 1960; McLean and Watson 1992). Cooper (1959, 1960) used three cycles of phenotypic selection for divergent maturity in two perennial ryegrass populations which led to variation far exceeding that of the original populations. In a recent RNA-Seq analysis, Zeng et al. (2017) identified a number of SNP markers of crested wheatgrass that could be used in the characterization of genes and pathways that are responsible for floral transition and development. These genomic resources can be useful in the development of marker-based tools for selecting late maturing genotypes in crested wheatgrass (Zeng et al. 2017).

2.4 Grass floral development

2.4.1 Process of floral induction and initiation

Moore et al. (1991) divided the life cycle of individual grass tillers into five primary growth stages: germination, vegetative, stem elongation, reproductive growth and seed (caryopsis) ripening stages. Heide (1994) defined floral induction as a "perception, transduction and transmittance of environmental signals resulting in plant developmental signals patterns from vegetative to reproductive." Increasing biomass yield potential in all grasses can be attained by manipulating the timing of floral transition (Colosanti and Coneva 2009; Purugganan and Fuller 2009). Floral induction stage is followed by a combination of both thermal and day length stimuli which cause the differentiation of the shoot apex from vegetative to floral development or reproductive phase (Heide 1994; Loeppky 1999). Srikanth and Schmid (2011) divided plants into three major groups on the basis of photoperiodic response: long–day plant flowers when day length exceeds a critical length (normally in the summer), short–day plant flowers when the day is short, and nights are long (normally in autumn), and day – neutral plant flowers without the influence of the length of the day. Blondon (1972) introduced the concepts of primary and secondary floral inductions to refer to these processes. To accelerate flowering, most temperate-region species may also have an additional requirement for vernalization which frequently substitutes for the short-day effect. Hence, primary floral induction of many temperate grasses is caused by short day and/or low temperatures with further floral development (secondary induction) caused or promoted by long days and warm temperatures (Evans 1987; Heide 1989). This is evident in crested wheatgrass which requires a two-step process for floral induction: exposure to short-day and cold temperature followed by exposure to long-day and warm temperatures in the following spring, these phases are followed by floral initiation and development internode elongation and seed filling (Heide 1994; Loepky 1999). However, no morphological changes are seen in most dual induction grasses such as crested wheatgrass until after the exposure to long-day and warm temperature. With respect to high latitude and high-altitude ecotypes, Habjorg (1978) reported that there is a need for such ecotypes to undergo both initiation and induction under short-day conditions but still requires long-day for flowering culm elongation. Many plants show precisely controlled seasonal patterns in flowering and it is only essential that flowering time is strictly regulated to ensure reproductive success and the completion of seed development in favorable environmental conditions (Andres and Coupland 2012).

Heide (1994) reported that exposure of temperate grasses to short-day during inflorescence development not only inhibits floral development, but also delays growth and developmental stages. In orchardgrass critical daylength for culm elongation was seen to be longer than daylength required for floral initiation (Niemelainen 1990).

2.4.1.1 Photoperiod

The ways in which plants respond to changes in day length (photoperiod) were first described in 1920 (Garner and Allard 1920).The response of a plant to length of day and night is termed as photoperiodic response (Song et al. 2015). Searle and Coupland (2004) explained that the ‘external coincidence model’ describes how exposure of a plant to light at a particular phase of a circadian rhythm would trigger or repress a developmental transition. Many plants use information about changing daylength (photoperiod) to align their flowering time with seasonal changes to increase reproductive success (Thomas and Vince-Prue 1997). Under long day conditions, the timing of flowering of *Arabidopsis* is accelerated through the function and the amount of FLOWERING LOCUS T (FT) protein under the direct control of the transcriptional activator CONSTANS (CO) protein (Srikanth and Schmid, 2011; Pin and Nilsson 2012; Pose et al. 2012; Andres and Coupland 2012). Flowering occurred much early under long day of 16 hr light than under short days of 10 hr light in *Arabidopsis* under laboratory conditions (Searle and Coupland 2004). The disruption in these responses was because of mutations, identification of mutants with a reduced response to day length was accomplished through isolation (Redie 1962; Koornneef et al. 1991). Koornneef et al. (1991) identified 11 loci (*fd, fwa, fe, fpa, fy, fve, ft, fha, fca, gi, co)* that resulted in late flowering time when mutated in an accession of *A. thaliana.* Four (*fca, fve, fy* and *fpa*) of 11 loci exhibited late flowering ability under both short and long-days when the flowering time of these mutants was subjected to different photoperiod and vernalization conditions.

2.4.1.2 Temperature

Besides photoperiod, temperature is another major determinant of flowering with fluctuations in temperature being sensed in the leaves of plants (Song et al. 2015). The word “vernalization” translated from the word “jarovization”; which was coined by a Russian scientist Lysenko in 1928 and was defined by Chourd in 1960 as “the acquisition or acceleration of the ability to flower by a chilling treatment” (Srikanth and Schmid 2011). Studies on the effects of temperature changes on flowering time have mostly focused on vernalization responses (Kim et al. 2009). A requirement for vernalization is an adaptive trait that helps prevent flowering before winter and permits flowering in the favorable conditions of spring. Kim et al. (2009) reported that, in *Arabidopsis* and cereals, vernalization results in the suppression of genes that repress flowering, and this is done by recruiting of chromatin-modifying complexes to a clade of flowering repressors that are silenced epigenetically via histone modifications. Napp-Zinn (1987) reported that a dominant locus called *FRIGIDA (FRI)* plays an important role in *Arabidopsis thaliana* as it grants a vernalization requirement to the natural accessions of this model plant in the analyses of the genetic differences between rapid cycling and winter-annual varieties. Further studies have shown that another gene called *FLOWERING LOCUS C (FLC)* in connection with *FRI* is both needed and necessary for vernalization to occur (Michaels and Amasino 1999; Koornneef et al. 1994; Lee et al. 1994). Geraldo et al. (2009) reported that the main function of *FRI* is to upregulate the expression of *FLC* which is a potent floral repressor. Sung and Amasino (2005) explained that, the epigenetic silencing of *FLC* in *A. thaliana* is by the activities of VERNALIZATION1 *(VRN1)*, VERNALIZATION2 *(VRN2)*, and VERNALIZATION INSENSITIVE3 *(VIN3)* genes. Srikanth and Schmid (2011) stated that vernalized plants become mitotically stable although genes are reset during meiosis since vernalization state cannot be passed from parents to progeny. Late flowering was observed in *Arabidopsis* when grown under LD conditions kept at the lower temperature of 16^{o}C than when grown at 23^{o}C with the difference primarily caused by differences in FT expression (Blazquez et al. 2003). Lee et al (2007) reported that the SHORT VEGETATIVE PHASE *(SVP)* gene mediates ambient temperature signaling in *Arabidopsis* and that the *SVP*-mediated control of *FLOWERING LOCUS T (FT)* expression is one of the molecular mechanisms evolved by plants to modulate the timing of the developmental transition to flowering phase in response to changes in the ambient temperature. High temperatures (i.e. 27 ^{o}C) also promote flowering with increased FT expression (Kumar et al. 2012; Proveniers and van Zanten 2013). Recent studies have indicated that Phytochrome Interacting Factor 4 (PIF4) is the main regulator for higher temperature-induced morphological changes, including floral transition (Proveniers and van Zanten 2013).

3.0 DNA content and ploidy determination of crested wheatgrass accessions using flow cytometry

3.1 Abstract

Crested wheatgrass [*Agropyron cristatum* L. (Gaertn.)] is widely used for early spring grazing in western Canada. The ploidy of crested wheatgrass varies from diploid to hexaploid. Ploidy determination is necessary before it can be effectively used in plant breeding and genetic studies. The objective of this study was to determine the ploidy of 45 crested wheatgrass accessions representing 18 different countries. On the basis of DNA content (pg $2C^{-1}$ = DNA content of diploid somatic nucleus), mean DNA content was 14.12 pg $2C^{-1}$ for diploid and 28.02 pg $2C^{-1}$ and 39.48 pg $2C^{-1}$ for tetraploid and hexaploid crested wheatgrass, respectively. Among the 45 accessions, there were eight diploid, 31 tetraploids, and six hexaploid accessions. With the exception accessions from Germany and Turkey, tetraploids were the most widespread as they were found in the remaining 16 countries. Diploid crested wheatgrass was found in germplasm from 6 countries (Canada, Germany, Kazakhstan, Romania, Sweden and United States), while hexaploid accessions mainly originated from Iran, Turkey, Russia and United States. This ploidy data provides important information for future marker trait analysis and cultivar improvement.

3.2 Introduction

Crested wheatgrass [*Agropyron cristatum* L. (Gaertn.)] is a long-lived, outcrossing caespitose, perennial grass widely seeded in the western Canada and USA (Copete et al. 2018). Due to its extensive fibrous root system and winter hardiness, crested wheatgrass is an excellent hay and pasture grass in the drier regions of temperate grasslands (Asay et al. 2003; Yu et al. 2012). It can be seeded to stabilize soil that have been contaminated by heavy metals (Miller and Dyer 2002; Meng et al. 2013; Guo et al. 2014).

Three main ploidy states exist within the crested wheatgrass complex (diploid, tetraploid, and hexaploid), which have the base chromosome number of n =7 (Dewey 1984; Asay and Jensen 1996). Even though forage yield and quality of crested wheatgrass have been successfully improved through breeding efforts in the last two decades (Asay et al. 2003; Gul et al. 2013), the ploidy of much of this germplasm is unknown. Previously, crested wheatgrass ploidy level has been determined for a limited number of cultivars (Yousofi and Aryavand 2004), but ploidy levels of a collection from diverse origin is necessary for the further genetic improvement of this species. In addition, grass populations with known ploidy levels are necessary for molecular marker development and gene identification (Wang et al. 2009).

Ploidy level has traditionally been determined by counting the number of chromosomes in a single plant cell (Yousofi and Aryavand 2004). This method has been used successfully in cactus and grain legumes species (Weedin and Powell 1978; Lackey 1980), but it is time consuming, laborious in species with a high number of chromosomes (De Laat et al. 1987; Rios at al. 2015). Attempts to determine ploidy level among plant species on the basis of morphological traits have been proven to be inaccurate (Wang et al. 2009). Alternatively, flow cytometry (FCM), is a useful technique to determine ploidy level and relative DNA content at a low cost (Ochatt 2006; Wang et al. 2009). Flow cytometry evaluates quantitative traits such as DNA and RNA content as well as cellular protein content with high precision and rapid throughput via the use of microscopy and biochemical analysis (Muirhead et al. 1985; Eaton et al. 2004). Several studies have demonstrated the usefulness of flow cytometry (FCM) in taxonomic studies. This technique is used to quantify the nuclear DNA content of a plant (2C value) and to infer the ploidy level using that value (Doležel et al. 2007). The interpretation of results in terms of ploidy is based on the study of nuclear DNA content (2C value) obtained from the analysis of both an unknown sample and a reference standard (Galbraith et al. 2001; Loureiro et al. 2006). Flow cytometry has been used to determine ploidy level and DNA content in many forage grasses such as perennial ryegrass (*Lolium perenne* L.) (Barker et al. 2001; Wang et al. 2009), Kentucky bluegrass (*Poa pratensis* L.) (Eaton et al. 2004), Bermudagrass (*Cynodon dactylon*) (Wu et al. 2006) and crested wheatgrass [*Agropyron cristatum* (L.) Gaertn] (Yousofi and Aryavand 2004).

The aim of this study was to determine DNA content and ploidy level of 33 crested wheatgrass accessions from USDA National Plant Germplasms System (NPGS), eight advanced breeding lines and four commercial cultivars using flow cytometry.

3.3 Materials and Methods

3.3.1 Plant Material

The crested wheatgrass germplasm used in this study included 33 accessions obtained from USDA National Plant Germplasm System (NPGS), eight advanced breeding lines from joint forage breeding programs between the University of Saskatchewan and Agriculture and Agri-Food Canada (AAFC) Saskatoon Research Center and four Canadian commercial cultivars (Table 3.1). The accession name, origin, and material status are shown in Table 3.1. Seedlings were grown in the greenhouse for eight weeks before being transplanted in the field nursery in July 2014 at the Agriculture and Agri-Food Canada Saskatoon Research Farm, Saskatoon (52° 07’ N, 106° 38’ W), SK, Canada. Row spacing was one meter between any two plants. Weed control was done by using triple rototiller (KS-190, Tram sales Ltd, AB) and by hand weeding around individual plants. Thirty-two individual plants for each accession were planted in the field using a randomized complete block design (RCBD) with four replications consisting of eight individual plants per replication. For each accession, eight individual plants (two plants from each replicate) were randomly selected for ploidy determination.

Table 3.1. Origin and material status information of the 45 crested wheatgrass accessions used in the study at Saskatoon SK, Canada

Accession	Country of Origin	Continent	Material status	Source[a]
AC Goliath	Canada	North America	Cultivar	Canadian Cultivar
AC Parkland	Canada	North America	Cultivar	Canadian Cultivar
Douglas	USA	North America	Cultivar	USDA NPGS
Kirk	Canada	North America	Cultivar	Canadian Cultivar
NewKirk	Canada	North America	Cultivar	Canadian Cultivar
Ruff	USA	North America	Cultivar	USDA NPGS
NU-ARD AC2	USA	North America	Cultivar	USDA NPGS
S9490	Canada	North America	Breeding line	U of S/AAFC joint program
S9512	Canada	North America	Breeding line	U of S/AAFC joint program
S9516	Canada	North America	Breeding line	U of S/ AAFC joint program
S9544	Canada	North America	Breeding line	U of S/AAFC joint program
S9556	Canada	North America	Breeding line	U of S/AAFC joint program
S9571	Canada	North America	Breeding line	U of S/AAFC joint program
S9580	Canada	North America	Breeding line	U of S/AAFC joint program
S9591	Canada	North America	Breeding line	U of S/AAFC joint program
Doneckij Sirokokolosyj	Ukraine	Europe	Cultivar	USDA NPGS
PI636511	Bulgaria	Europe	Germplasm	USDA NPGS
PI547351	France	Europe	Germplasm	USDA NPGS
PI281862	Germany	Europe	Germplasm	USDA NPGS
PI494616	Romania	Europe	Germplasm	USDA NPGS
PI439914	Russia	Europe	Germplasm	USDA NPGS
PI547286	Russia	Europe	Germplasm	USDA NPGS
PI564869	Russia	Europe	Germplasm	USDA NPGS
PI564879	Russia	Europe	Germplasm	USDA NPGS
PI564880	Russia	Europe	Germplasm	USDA NPGS
PI406442	Russia	Europe	Germplasm	USDA NPGS
PI318922	Spain	Europe	Germplasm	USDA NPGS
PI297869	Sweden	Europe	Germplasm	USDA NPGS
PI173622	Turkey	Europe	Germplasm	USDA NPGS
PI628683	Ukraine	Europe	Germplasm	USDA NPGS
PI316120	Australia	Australia	Germplasm	USDA NPGS
PI662330	Armenia	Asia	Germplasm	USDA NPGS
PI499390	China	Asia	Germplasm	USDA NPGS
PI401076	Iran	Asia	Germplasm	USDA NPGS
PI401080	Iran	Asia	Germplasm	USDA NPGS
PI401085	Iran	Asia	Germplasm	USDA NPGS
PI401086	Iran	Asia	Germplasm	USDA NPGS
PI598641	Kazakhstan	Asia	Germplasm	USDA NPGS
PI639849	Kazakhstan	Asia	Germplasm	USDA NPGS
W625134	Kazakhstan	Asia	Germplasm	USDA NPGS
PI628672	Mongolia	Asia	Germplasm	USDA NPGS
PI639815	Mongolia	Asia	Germplasm	USDA NPGS
PI547346	China	Asia	Germplasm	USDA NPGS
PI670374	China	Asia	Germplasm	USDA NPGS
PI516482	Morocco	Africa	Germplasm	USDA NPGS

[a]Source, U of S= University of Saskatchewan; USDA NPGS= United States Department of Agriculture National Plant Germplasm System

3.3.2 Ploidy determination

At early vegetative growth stage in 2017, two young, fresh leaf tissues of eight randomly selected individual plants were harvested from each of the 45 accessions and placed in a labeled, plastic zip lock bag. The collected leaf tissues were placed on ice in a Styrofoam cooler during transportation from the field to lab.

To calibrate and set the gain on the ploidy analyzer to match the material being tested, AC Parkland, a known diploid crested wheatgrass was found to reliably produce peaks and hence was used as an internal control.

For the preparation of suspensions of intact nuclei, approximately 1 cm^2 of leaf was excised from the leaf blade and finely chopped with razor blade for 30 to 60 s to release nuclei in a 60 x 15mm standard sterilized, disposable plastic petri dish (Fisher Scientific, USA) , containing 1.5ml of commercial Partec DAPI (4'6-diamidino-2-phenylindole) staining buffer. DAPI was used as a staining agent as it is an effective fluorochrome for determining ploidy level (Burson et al. 2012). Flow cytometry analysis was performed using Partec CyFlow Space (Partec GmbH, Germany) equipped with UV light and 455BP filter. The ploidy analyzer was adjusted to channel 50 which is the G1 (gap between mitosis and the onset of DNA synthesis) peak of the nuclei isolated from the diploid internal control. Samples for analysis were then run through the cytometer. The calibration was checked periodically and kept constant between runs to minimize variation. To estimate ploidy level, position of the G1 peak of histograms (Figure 3.1 A-C) obtained for each sample was compared to that of the internal control (AC Parkland). This was done using Partec software package. The ploidy of each crested wheatgrass accession was determined using the FloMax software (Partec GmbH, Munster, Germany). Four plants of each accession were individually evaluated for ploidy level. In situations where the results from an accession were in question, an additional four plants were examined. The DNA content of the samples was determined by the equation: nuclear DNA content = (mean position of sample peak)/ mean position of control peak) x DNA content of the control (Yousofi and Aryavand 2004; Wang et al. 2009; Copete et al. 2018).

3.4 Results

3.4.1 Ploidy level among crested wheatgrass accessions

The ploidy level and the mean nuclear 2C DNA content of the 45 crested wheatgrass accessions is given in Table 3.2. The average DNA content (2C) for diploid (2n=14), tetraploid (2n=28), and hexaploid (2n=42) were 14.12, 28.02 and 39.48 pg, respectively.

Table 3.2 Nuclear DNA content, and ploidy of 45 crested wheatgrass determined by flow cytometer evaluated in the study at Saskatoon SK Canada

Continent	Accession	Origin	Ploidy[a]	Mean 2C DNA content (pg) ±SD[b]
North America	AC Goliath	Canada	4x	27.49 ± 0.57
North America	Kirk	Canada	4x	27.93 ± 0.28
North America	AC Parkland	Canada	2x	13.83 ± 1.08
North America	New Kirk	Canada	4x	27.94 ± 1.21
North America	S9490	Canada	4x	27.94 ± 0.66
North America	S9512	Canada	4x	27.83 ± 0.90
North America	S9516	Canada	4x	27.13 ± 1.20
North America	S9544	Canada	4x	27.55 ± 0.81
North America	S9556	Canada	4x	27.37 ± 1.04
North America	S9571	Canada	4x	27.79 ± 2.07
North America	S9580	Canada	4x	27.96 ± 1.26
North America	S9591	Canada	4x	27.82 ± 0.51
North America	PI 578519	USA	2x	14.07 ± 0.48
North America	PI 634507	USA	2x	14.82 ± 0.44
North America	Douglas	USA	6x	39.74 ± 1.95
Europe	PI 636511	Bulgaria	2x	13.87 ± 0.37
Europe	PI 547351	France	4x	27.97 ± 0.37
Europe	PI 281862	Germany	2x	14.19 ± 0.47
Europe	PI 494616	Romania	2x	14.25 ± 0.70
Europe	PI 439914	Russia	4x	28.09 ± 1.17
Europe	PI 547286	Russia	4x	28.51 ± 0.95
Europe	PI 564869	Russia	4x	26.31 ± 0.91
Europe	PI 564879	Russia	4x	28.03 ± 0.89
Europe	PI 564880	Russia	4x	27.31 ± 0.94
Europe	PI 406442	Russia	6x	38.45 ± 0.20
Europe	PI 318922	Spain	4x	27.53 ± 1.49
Europe	PI 297869	Sweden	2x	14.18 ± 0.02
Europe	PI 173622	Turkey	6x	39.63 ± 0.43
Europe	PI 486163	Ukraine	4x	28.49 ± 0.85
Europe	PI 628683	Ukraine	4x	27.61 ± 1.38
Australia	PI 316120	Australia	4x	28.21 ± 1.74
Asia	PI 662330	Armenia	4x	29.48 ± 1.80

Asia	PI 499390	China	4x	29.18 ± 1.05
Asia	PI 401076	Iran	6x	40.59 ± 0.29
Asia	PI 401080	Iran	6x	39.13 ± 1.04
Asia	PI 401085	Iran	6x	39.33 ± 1.02
Asia	PI 401086	Iran	4x	28.75 ± 2.95
Asia	PI 598641	Kazakhstan	4x	27.63 ± 1.11
Asia	PI 639849	Kazakhstan	2x	13.72 ± 0.73
Asia	W625134	Kazakhstan	4x	27.12 ± 0.77
Asia	PI 628672	Mongolia	4x	27.70 ± 1.75
Asia	PI 639815	Mongolia	4x	29.29 ± 0.82
Asia	PI 547346	China	4x	28.23 ± 0.64
Asia	PI 670374	China	4x	29.91 ± 1.43
Africa	PI 516482	Morocco	4x	28.06 ± 1.05

[a]Ploidy, 2x = diploid; 4x = tetraploid; 6x = hexaploid. [b]S.D, Standard deviation

Flow cytometry fluorescence histograms of representation examples of each ploidy level are shown in Figure 3.1 A-C. The 45 crested wheatgrass populations were grouped into three ploidy levels. There were eight diploid, 31 tetraploid and six hexaploid populations (Table 3.3).

Table 3.3 Ploidy level of crested wheatgrass by country and continent evaluated in the study at Saskatoon SK Canada

Country	Number of populations	Ploidy level[a]
Armenia	1	4x
Australia	1	4x
Bulgaria	1	4x
Canada	12	2x, 4x
China	3	4x
France	1	4x
Germany	1	2x
Iran	4	4x, 6x
Kazakhstan	3	2x, 4x
Mongolia	2	4x
Morocco	1	4x
Romania	1	2x
Russia Federation	5	4x
Former Soviet Union	1	6x
Spain	1	4x
Sweden	1	4x
Turkey	1	6x
Ukraine	2	4x
United States	3	2x, 6x
Continent	5	
North America	15	2x, 4x, 6x
Europe	15	2x, 4x, 6x
Asia	13	2x, 4x, 6x
Africa	1	4x
Australia	1	4x

[a]Ploidy, 2x = diploid; 4x = tetraploid; 6x = hexaploid

Twenty nine of 45 accessions were wild collections from their respective countries. Tetraploid crested wheatgrass was the most widely distributed type found in 12 of 18 countries of origin. Diploid types were found in 8 countries, while the hexaploid accessions were from four countries (Iran, Former USSR, Turkey and United States). Except for AC Parkland, all modern cultivars and breeding lines of Canada were tetraploid. By country of origin, single ploidy level was found for 14 of 18 countries, and two forms of ploidy were observed in four countries (Canada, USA, Kazakhstan, and Iran), but no single country had all three forms in our study. By continent, Asia, Europe, and North America have diploid, tetraploid, and hexaploid crested wheatgrass germplasms (Table 3.3).

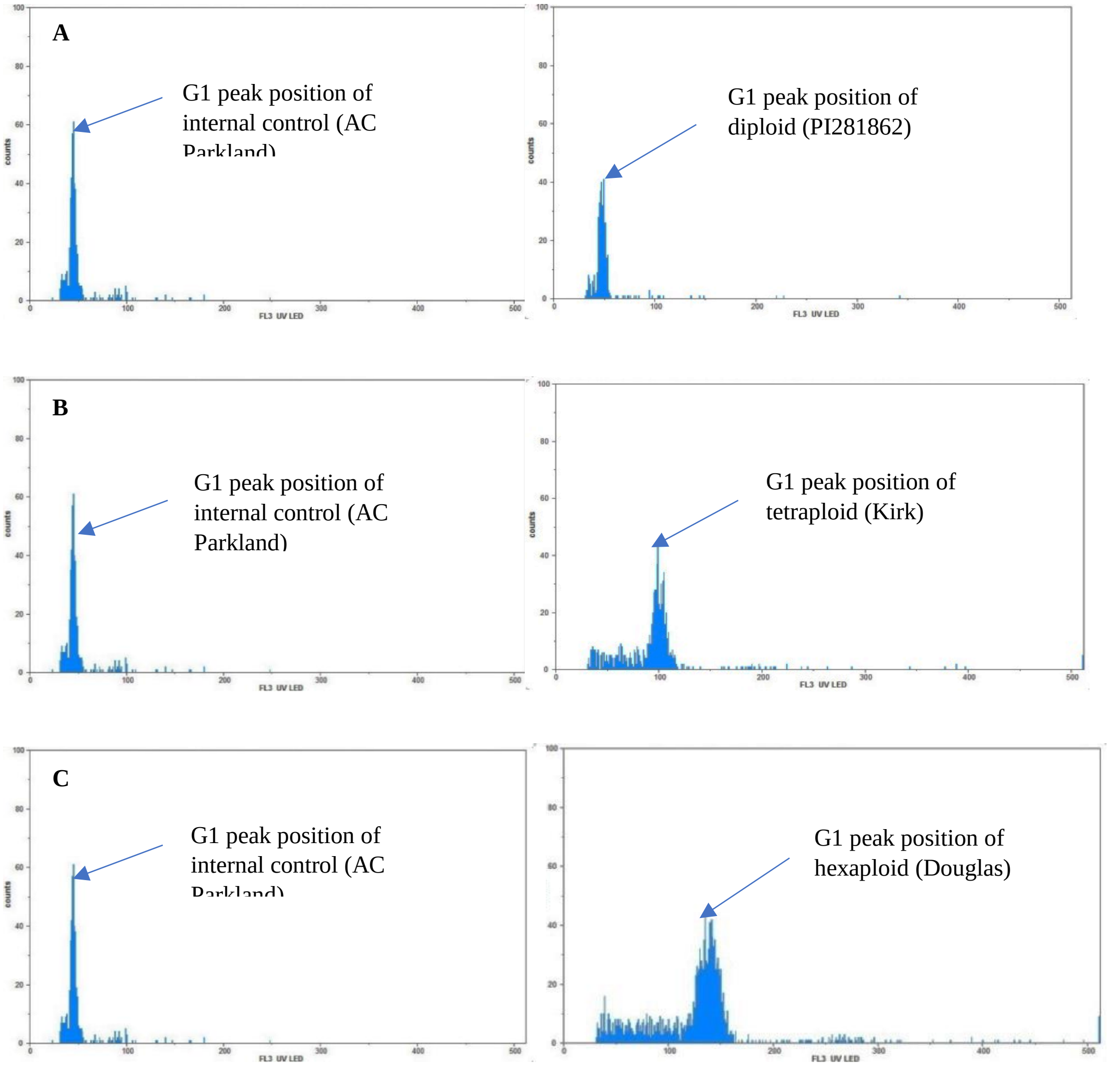


Figure 3.1 Flow cytograms of 3 crested wheatgrass accessions with different ploidy levels: **A)** diploid, **B)** tetraploid and **C)** hexaploid with AC Parkland as control

3.5 Discussion

Flow cytometry proved to be an effective and highly reliable methodology for characterizing ploidy level of crested wheatgrass germplasm. In this study, ploidy level of all 45 crested wheatgrass accessions were determined, which is invaluable to crested wheatgrass genetic and genomic studies. Crested wheatgrass complex contains germplasm with three ploidy levels, namely diploid (2n = 14), tetraploid (2n = 28) and hexaploid (2n = 42) (Dewey 1984; Yu et. al.

2012; Zeng et. al 2017). The mean nuclear DNA content (2C value) obtained for diploid, tetraploid and hexaploid accessions in this study was in agreement to earlier studies conducted for diploid accessions (Vogel et al. 1999), both tetraploid and hexaploid accessions (Yousofi and Aryavand 2004), diploid, tetraploid and hexaploid accessions (Copete et al. 2018). In this study, the three ploidy levels were detected, and, among the 45 crested wheatgrass accessions , diploid, tetraploid and hexaploid accounted for 17.8%, 68.9% and 13.3%, respectively. Tetraploid crested wheatgrass was found in accessions from North America, Europe and Asia as well as from Morocco and Australia. Copete et al. (2018) found that among the three crested wheatgrass ploidy level, tetraploids were the most widely distributed type ranging from Europe, Morocco, and the Middle East across Central Asia to Siberia, China and Mongolia. Tetraploid populations exist in the natural population of crested wheatgrass, but artificial colchicine induced tetraploids have also been developed. ‘AC Goliath’ a tetraploid cultivar originates from colchicine-doubled plants of the diploid *A. cristatum* cultivar ‘AC Parkway’ and plants from the natural tetraploid *A. cristatum* cultivar ‘Kirk’ (Coulman 2006). Tetraploids generally are generally larger, taller than diploids, with upright tillers (Mellish and Coulman 2002). Use of hexaploid crested wheatgrass is limited. ‘Douglas’ is the first hexaploid crested wheatgrass cultivar to be released from USDA-ARS Forage Research and Range Research (FRR) Laboratory (Ogle 2006). It is characterized by having broad leaves, being leafier (high leaf-to-stem ratio), and its leaves remain green for longer period during the growing season than other crested wheatgrass cultivars (Asay et al. 1995). With increased leafiness being an important breeding goal, the use of hexaploids in the breeding programs may also be useful in helping produce crested wheatgrass lines with higher leaf-to-stem ratio at mature growth stages.

Geographic distribution has been reported to have a relationship with ploidy levels of crested wheatgrass (Asay and Dewey 1979; Dewey and Asay 1982; Dewey 1984). In the current study, diploid accessions were found in North America (Canada and United States), Europe (Bulgaria, Germany, Romania and Sweden) and Asia (Kazakhstan), however no diploid accessions were found from Africa or Australia with the exception of the cultivar 'Douglas' (USA), natural populations of hexaploids were mainly found in Iran, Turkey, and Russia. 'Douglas' was derived from hybrid between an accession from former Soviet Union (PI406442) and four other accessions from Iran (PI401076, PI401080 and PI401085) and one additional accession from Turkey(PI173622) (Asay et al. 1995). With the exception of Turkey, Iran and Georgia, diploids have been reported to spread in small areas being distributed over the same general range as tetraploid (Copete et al. 2018) in agreement with previous reports (Yousofi and Aryavand 2004; Copete et. al 2018). In a genetic characterization of *A. cristatum* using SSR markers, Che et al. (2008) found hexaploid accessions in the northern China, but due to our sample size (three accessions from China), we did not identify any hexaploid originating from China.

All three forms of crested wheatgrass are economically important, and many cultivars were released in the past (Asay et al. 1995, 2003; Asay and Jensen 1996). Even though there is a clear distinction among the three forms, inter-ploidy hybridization is possible (Dewey 1984). The genetic introgression that exist between three ploidy levels of crested wheatgrass means that the 2x, 4x, and 6x accessions of crested wheatgrass should be treated as a common gene pool (Dewey 1984; Copete 2018) . The potential of combining the genetic resources of diploid, tetraploid, and hexaploid through interploidy hybridization involving 6x-2x, 6x-4x, 4x-2x is useful for expanding the genetic resources and specific trait introgression such as the broad leaf of hexaploids to tetraploid types (Dewey 1969, 1973; Asay and Dewey 1979; Asay et al. 1995; Jensen et al. 2005).

In summary, the ploidy level of 45 crested wheatgrass accessions with diverse origins were successfully identified using flow cytometry. Eight diploids, 31 tetraploids and six hexaploids were identified. The average DNA content was 14.12 pg $2C^{-1}$ for diploids, 28.02 pg $2C^{-1}$ for tetraploids and 39.48 pg $2C^{-1}$ for hexaploids. Determining ploidy level in crested wheatgrass helps increase precise crossing within ploidy levels or promotes ploidy hybridization to increase variation for specific traits such as leafiness and plant maturity. Identification of diploid type though flow cytometry could be useful for assembling mapping populations to analyze genetic diversity and population structure or validating genomic selection models as high ploidy levels increase challenges for bioinformatics analysis.

4.0 Assessment of crested wheatgrass (*Agropyron cristatum* L.) accessions with different geographical origins for agronomic and phenotypic traits and nutritive value

4.1 Abstract

The objectives of this study were to characterize crested wheatgrass (*Agropyron cristatum* L.) germplasm for plant maturity and associated agronomic characteristics to identify superior germplasm with late maturity. A field trial was established in July 2014 at Saskatoon, Canada with 45 crested wheatgrass accessions representing materials from 18 countries using a randomized complete block design with four replications. Data were collected for days to heading, plant height, leaf-to-stem ratio, forage dry matter (DM) yield, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and plant vigor score in 2015, 2016 and 2017. All measured traits differed significantly ($P \leq 0.05$) among the accessions. Later maturing accessions showed positive associations with leaf-to-stem ratio (r=0.23), NDF (r=0.16), ADF (r=0.18), but had negative correlations with CP (r=-0.30) and spring vigor (r=-0.11). Forage DM yield was positively correlated with spring vigor (r=0.77), leaf-to-stem ratio (r=0.50), plant height (r=0.56), regrowth (r=0.67), hay stage vigor (r=0.58), leafiness (r=0.79), and ADF (r=0.11). There was a negative correlation between forage DM yield and CP concentration (r=-0.23). According to the unweighted pair group method with arithmetic mean (UPGMA) and principal component analysis (PCA), the 45 crested wheatgrass accessions were grouped into three main clusters according to the agro-morphological and nutritive value data. Accessions in Cluster I were tall, and produced high forage DM yield. Accessions in Cluster II had high CP and low ADF and NDF concentrations. Accessions in Cluster III had late plant maturity and high leaf-to-stem ratio. Selection for late maturity in crested wheatgrass may lead to increase in leaf-to-stem ratio, which may also increase forage DM yield as it was positively related to leafiness

4.2 Introduction

Crested wheatgrass (*Agropyron cristatum* L.) is a drought tolerant perennial, caespitose grass with an extensive root system adapted to the semi-arid and arid regions of the Northern Great Plains (Yu et al. 2012; Iwaasa et al. 2014). Due to its good forage quality and palatability, crested wheatgrass is a preferred grass for cattle, sheep, and horses (Li et al. 2004). Crested wheatgrass is important for early spring grazing in western Canada because of its early growth. However, it also reaches mature growth stage early, and mature plants are coarse and unpalatable for grazing cattle. The mature plants also showed a low leaf-to-stem ratio and high fiber concentration (Daugherty et al. 1982).

Characterization of agro-morphological traits of plant germplasm collections is important for the estimation of genetic variability among and within plant populations as well as providing a baseline phenotypic and agronomic information (Fufa et al. 2005). In crested wheatgrass, characterization of germplasm for agro-morphological traits has not been extensively conducted compared to other forage crops such as sainfoin (*Onobrychis viciifolia* Scop.) (Bhattarai et al. 2018), white clover (*Trifolium repens* L.) (Lane et al. 2000), alfalfa (*Medicago sativa* L.) (Warbuton and Smith 1993; Smith et al. 1995; Abbasi et al. 2006 2007; Basafa and Taherian 2009). To develop a later maturing cultivar of crested wheatgrass with high nutritional quality, this study evaluated agro-morphological characteristics and nutritive value of 45 crested wheatgrass accessions originating from Africa, Asia, Australia, Europe, and North America under western Canadian ,growing conditions.

4.3 Materials and Methodology

4.3.1 Field experiment

4.3.1.1 Plant material

A total of 45 crested wheatgrass accessions representing different geographical origins were used for this study (Table 3.1). These accessions consisted of eight advanced breeding lines from joint breeding programs between the University of Saskatchewan and Agriculture and Agri-Food Canada (AAFC) Saskatoon Research Center, 33 crested wheatgrass introductions from the National Plant Germplasm System (NPGS) of United State. Four Canadian crested wheatgrass cultivars: Kirk, Newkirk, AC Parkland and AC Goliath were included for comparison.

4.3.1.2 Experimental design

A field trial was established using 45 crested wheatgrass accessions in July 2014 at Agriculture and Agri-Food Canada (AAFC) Saskatoon Research Center, Saskatoon, SK (52^{o} 07’ N, 106^{o} 38’ W) (Figure 4.1A). The experimental design was a randomized complete block design (RCBD) with four replications. The total number of plants was 1440 (45 accessions x 8 plants plot^{-1} x 4 replicates). Seedlings were grown in the greenhouse for eight weeks before transplanting into the field. Spacing between rows and plant within a row was 1 m. Weed control was done using a triple rototiller (KS-190, Tram sales Ltd, AB) in early spring in addition to hand weeding around individual plants. The soil was a Sutherland clay loam (Dark Brown Chernozem, Typic Haploloroll) (Acton and Ellis 1978). Weather data for Saskatoon from 2014 to 2017 was obtained from Environment Canada (climate.weather.gc.ca).

4.3.1.3 Agro- morphological data collection

Agro-morphological traits and corresponding measurement methods are shown in Table 4.1. Data were collected for spring vigor (Figure 4.1B), plant height (Figure 4.1C), days to heading (Figure 4.1D), leaf-to-stem ratio (Figure 4.1E), hay stage vigor, forage DM yield and regrowth during summers of 2015, 2016 and 2017. Leafiness score, and crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations were determined only in 2016 and 2017.

4.3.1.4 Forage nutritive value

Plants were sampled at the early anthesis stage in 2016 and 2017 and dried at 60°C for 48 h in a forced-air oven. The dried plant samples were grounded in a Wiley mill (Thomas-Wiley, Philadelphia, PA) and then passed through a 1-mm mesh screen (Cyclone Mill, UDY Mfg., Fort Collins, CO). Ground samples were stored in filter bags (Nasco Whirl-Pak, USA) prior to CP, ADF and NDF determinations. For crude protein determination, nitrogen content in the ground samples was analyzed using a Leco 628 Element Analyzer (Leco Corporation, St. Joseph, MI). Approximately 0.1g ground sample was wrapped in tin foil into a capsule-like shape. In the Leco 628 Element Analyzer, each sample was combusted with oxygen, and the gases containing nitrogen oxides were collected in a ballast tank until a specified pressure was reached. Helium gas was used as a carrier and an aliquot of combustion gas containing nitrogen oxides was reduced to nitrogen. It was then passed through a tube containing magnesium perchlorate and sodium hydroxide to remove water and carbon dioxide. Nitrogen was measured with a thermal conductivity detector using helium as a reference. Nitrogen content was converted to crude protein by multiplying by a conversion factor 6.25.

Ground samples were analyzed for ADF concentration using the Ankom^{2000} automated fiber analyzer (ANKOM Technology Corporation, New York). For this procedure, a labeled F57 filter bag (ANKOM Technology, Corporation, New York) was weighed and recorded (W1). A small quantity (0.4-0.5g) of the ground sample was weighed and recorded (W2). Then, filter bags with samples were sealed using the heat sealer. The weight of one blank bag was measured and recorded to determine blank bag correction (C1). Three bags were placed on each of the eight trays (24 bags in total) with the ninth tray kept empty. The empty ninth tray was placed on top. The bag suspender was inserted into the fiber analyzer vessel at an internal temperature of 20 °C. During analysis, cell contents were removed as the encapsulated sample was subjected to an acid detergent solution digestion for 1.5 h. After ADF digestion, filter bags were washed in a 250ml beaker using acetone for 3-5 min. The samples were dried under the fume hood for 6 min, followed by drying in an oven at 102° C for 4h. Then, samples were cooled to room temperature, weighed and recorded (W3).

ADF was calculated as:

$$= \frac{100 \times [(W3-(W1xC1)]}{W2}$$

Ground samples were analyzed sequentially for NDF concentration using the Ankom^{2000} automated fiber analyzer (ANKOM Technology Corporation, New York). The procedure for NDF analysis is similar to ADF analysis except NDF solution with 20g of sodium sulfite was added to the sample followed by a 2h digestion process.

Table 4.1 Agro-morphological trait analysis of 45 crested wheatgrass accessions

Trait	Method of measurement	Date of measurement
1. Spring vigor	visually scored for 1-5 scale, 1= poor plant vigor, 5= vigorous plant growth	May 8 2015 May 6 2016 May 10 2017
2. Plant height (cm)	Measured as the height of the tallest reproductive culm near the center of the plant	June 3 – 15 June 2015 May 26 – June 13 2016 May 29 – June 15 2017
3. Days to heading	Measured from May 1 to the date for the first head emergence and expressed using growing degree days (GDD). GDD was calculated based on Frank and Hofmann (1989)	May 1 – June 17 2015 May 1 – June 13 2016 May 1– June 15 2017
4. Leafiness	visually scored for 1-5 scale, 1 = low percent of leaves, 5 = high percent of leaves	June 8 2016 June 12 2017
5. Hay stage vigor	visually scored for 1-5 scale, 1 = plants with a low biomass yield, 5 = plants with a high biomass yield	June 20 2015 June 8 2016 June 12 2017
6. Leaf-to-stem ratio	Randomly selected three plants per population in each replication, and 10 tillers plant^{-1} were used for leaf-to-stem ratio determination. The leaves and stems were hand separated and dried at 60°C for 48 h in a forced air oven. Then, the stem and leaf dry matter weights were determined and leaf-to-stem ratio for each accession was calculated.	June 15 – 20 2015 June 17 – 29 2016 June 15 – 30 2017
7. Forage (DM) yield (g plot^{-1})	After fresh forage yield measurement, a sub-sample was taken from each plot for moisture content (%) determination by drying at 60°C for 48 h. Then, total forage DM yield was calculated.	July 10 2015 June 24 2016 July 10 2017
8. Regrowth	visually scored for 1-5 scale, 1 = slow regrowth, 5 = rapid growth	August 10 2015 July 29 2016 August 7 2017

4.3.2 Growth chamber experiment

4.3.2.1 Evaluation of heading date of selected crested wheatgrass accessions under controlled growth environment

To further evaluate flowering time in selected germplasm of crested wheatgrass under a controlled environment, six accessions consisting of two early maturing accessions [PI439914 (351 GDD) from Ukraine and PI516482 (352 GDD) from Morocco], two late maturing accessions [S9580 (441 GDD) from Canada and PI401085 (467 GDD) from Russia] and two Canadian cultivars [Kirk (367 GDD) and AC Parkland (415 GDD] were selected for this study. Selection of the accessions was based on average days to heading in the field experiment. A total of 24 genotypes representing the six selected accessions were dug from the experimental field plot at Agriculture and Agri-Food Canada Saskatoon Research Station in November 2017 after vernalization in the field. All plants were stored at -18^{o}C prior to being split into four clones per plant.

The experiment was conducted in growth cabinets in the phytotron of the College of Agriculture and Bioresources at the University of Saskatchewan using genotypes from each of five of the accessions described above. Accession PI401085 did not grow during the first run of the experiment, hence it was removed from the study. The experimental design was a randomized complete block design (RCBD) with two replications. In each replication, there were five crested wheatgrass accessions, with four genotypes from each accession, and each genotype having two identical clones. Plants in the growth chamber were subjected to 16/8 h day/night at 22/16^{o}C, and light was provided by fluorescent cool white bulbs (T50 HO 835; 3500k) and the whole experiment was replicated twice from February 22 – April 13, 2017 and from July 13 – September 29, 2017.

After the first run of the experiment, plants were stored in a 4^{o}C room for vernalization. All plants were fertilized using 20-20-20 (N: P: K) nutrient solution once every week for three weeks. Data on plant height and tiller number per plant at heading, and days to heading were recorded. Plant height was measured as the height of the tallest reproductive culm near the center of the plant. Tiller number at heading was recorded by counting all tillers. Days to heading was recorded as total number of days required for first head emergence.

4.4 Statistical Analyses

An analysis of variance (ANOVA) was performed among 45 crested wheatgrass accessions for each trait using the Mixed Model procedure of Statistical Analysis System software (SAS 9.4 SAS Institute 2014). Accessions, year and their interaction were considered as fixed effects while replication was taken as a random effect. For each trait, if the ANOVA indicated significant differences at the $P \leq 0.05$ level, the means were separated using the least significant difference (LSD) method. Values of measured traits were averaged across the three years if no significant year x accession ($P > 0.05$) was detected.

Data for each year were analyzed separately due to significant year × accession interactions for most measured traits. For analysis of individual years, accession was considered as a fixed effect and replication was considered as a random effect. In each analysis, if the ANOVA indicated significant differences at the $P \leq 0.05$ level, the means were compared by calculating the least significant differences (LSD) at $P \leq 0.05$. Pearson correlation coefficients were calculated using the CORR procedure of SAS (SAS 9.4 SAS Institute 2014).

The Euclidean distance matrix of the 45 accessions using the means for 11 agro-morphological and nutritive value traits across three years was constructed using the R software package, version 3.3.2 (R Core Team 2016). Clustering of accessions using the Euclidean distance matrix was performed by SAHN in NTSYS-pc 2.1 using the unweighted pair group method with arithmetic mean (UPGMA) (Rohlf 1997). Pearson correlation coefficients were determined and principal component analysis (PCA) was conducted in SigmaPlot 13.0 software for all measured traits.

An analysis of variance (ANOVA) was performed to compare three ploidy groups of the 45 crested wheatgrass populations using the Mixed Model procedure of Statistical Analysis System software (SAS 9.4 SAS Institute 2014). Ploidy levels were assumed to be a fixed effect and year, replication and their interaction were assumed to be random effects in the model and significance was declared at $P \leq 0.05$. In each analysis, if the ANOVA indicated significant differences at the $P \leq 0.05$ level, the means were compared by calculating a least significant difference (LSD) at $P \leq 0.05$.

For the growth chamber experiment, analysis of variance (ANOVA) was performed among the five selected crested wheatgrass accessions for each trait using the Mixed Model procedure of Statistical Analysis System software (SAS 9.4 SAS Institute 2014). Accessions were considered as fixed effects while replication, run and their interaction were taken as a random effect in the model and significance were declared at $P \leq 0.05$. In each analysis, if the ANOVA indicated significant differences at the $P \leq 0.05$ level, the means were compared by calculating least significant difference (LSD) at $P \leq 0.05$.

4.5 Environmental conditions

The average monthly air temperature and precipitation at the study site are shown in Table 4.2.

Table 4.2 Monthly mean temperature and precipitation during 2014, 2015, 2016 and 2017, and long-term average monthly temperature and precipitation in Saskatoon SK, Canada

	Monthly mean temperature					Monthly precipitation				
	2014	2015	2016	2017	30-yr[a]	2014	2015	2016	2017	30-yr
Month	----------------------------		°C	------------------------------		---------------------------		mm	----------------------------	
January	-15.0	-11.8	-12.9	-13.1	-13.9	6.1	5.8	17.3	7.4	14.6
February	-19.2	-17.4	-7.9	-9.3	-11.4	2.1	16.5	7.0	9.1	9.1
March	-10.1	-2.4	-1.5	-5.2	-4.9	5.8	5.1	13.9	11.3	14.5
April	-1.7	5.6	5.5	4.3	5.2	74.2	21.1	3.0	18.4	21.8
May	10.1	10.1	13.7	12.1	11.8	61.1	0.4	41.6	46.3	36.5
June	14.1	17.2	17.4	16.1	16.1	94.8	13.6	49.7	30.9	63.6
July	18.3	19.4	18.7	19.6	19.0	44.5	84.3	58.6	25.5	53.8
August	17.9	17.4	16.9	17.8	18.2	18.5	45.2	70.2	25.2	44.4
September	12.4	11.9	11.8	12.8	12.0	10.7	50.0	24.1	29.1	38.1
October	6.7	6.7	2.1	5.0	4.4	14.1	33.9	40.8	17.8	18.8
November	-9.7	-3.1	1.9	-24.8	-5.2	30.5	14.0	9.2	15.4	12.4
December	-9.4	-9.3	-13.7	-12.3	-12.4	2.5	2.5	9.7	6.9	12.8
Total	-	-	-	-	-	364.9	292.4	345.1	243.3	340.5

[a] 30 yrs (1981-2010) mean value obtained from Environment Canada (2018).

Growing season (May to September) air temperature during the 4-yr study was close to the long-term mean. In the stand establishment year in 2014, the total precipitation was above the long-term average. Almost no rainfall was recorded during May and June in 2015, which negatively affected growth and development of crested wheatgrass in our study. In 2016, total precipitation was almost the same as the long-term average. growth. Even though the total precipitation in 2017 was much lower than the long-term average, there wasn't any significant effect on the growth of crested wheatgrass.

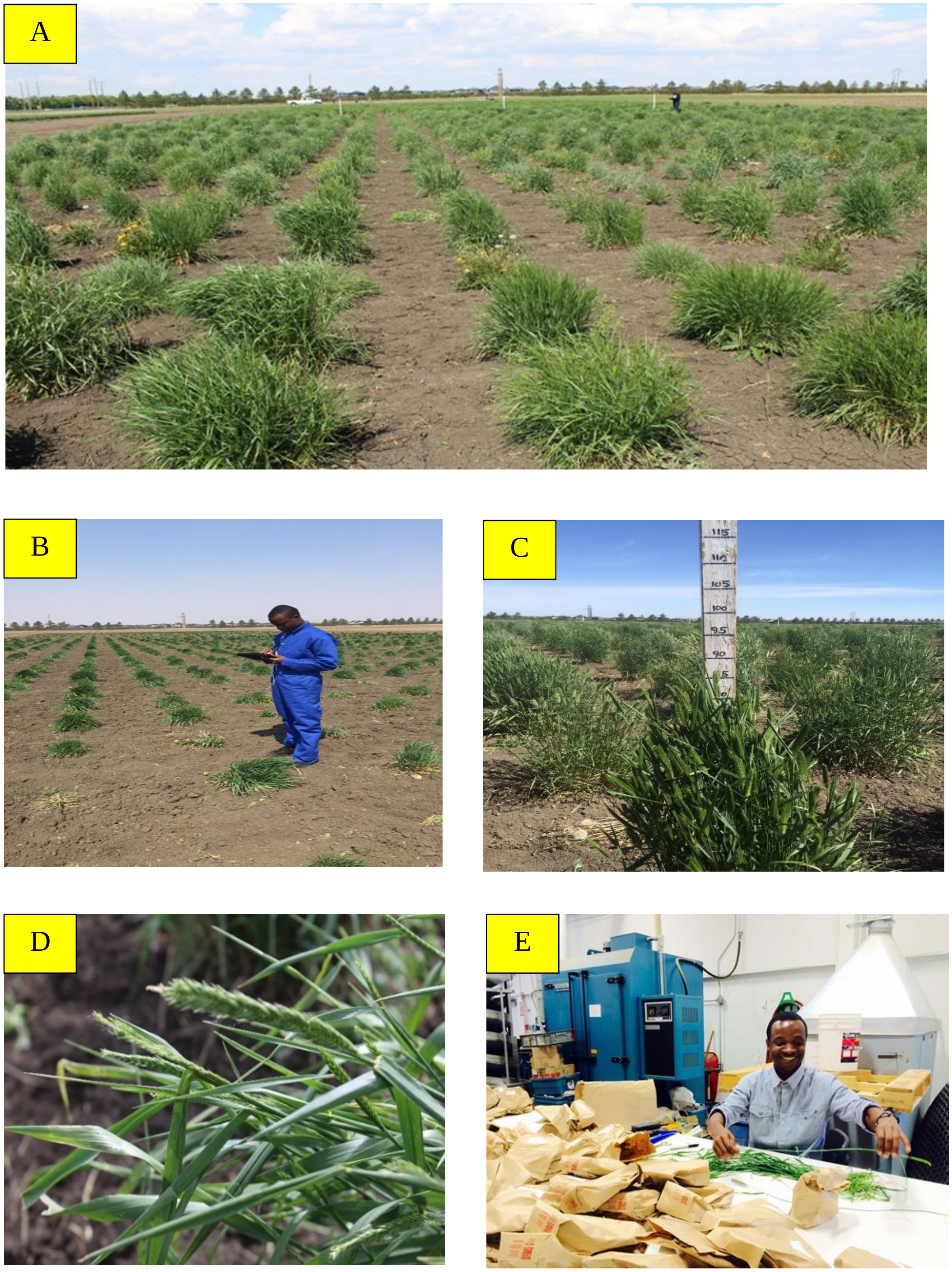


Figure 4.1 A) Crested wheatgrass research plot at Saskatoon B) Spring vigor scoring C) Plant height measurement at heading D) Head emergence of crested wheatgrass E) Plant leaf and stem separation.

4.6 Results

4.6.1 Variation in agro-morphological traits and nutritive value of 45 crested wheatgrass accessions

There were significant ($p < 0.05$) differences among the crested wheatgrass accessions in all measured traits (Table 4.3). The agro-morphological and nutritive value traits significantly ($p < 0.05$) differed among the three years except for NDF concentration. Year x accession interaction was significant ($p < 0.05$) for spring vigor, regrowth, hay stage vigor, days to heading, DM yield, plant height, leaf-to-stem ratio, and crude protein, thus data were reported by the individual year (Table 4.3). Year x accession interaction was not significant for leafiness score, ADF and NDF, thus, data were averaged across the three years for each accession for these three traits (Table 4.3).

Table 4.3 *F* values from the analysis of variance of 45 crested wheatgrass evaluated for 11 traits in 2015, 2016 and 2017 in Saskatoon SK, Canada

Source	df[a]	SV	RG	HSV	LFS	DH	DMY	PH	LSR	CP	NDF	ADF
Accession	44	35.27 ***[b]	25.19 ***	6.29 ***	12.65 ***	15.55 ***	15.00 ***	40.18 ***	5.14 ***	2.57 ***	3.46 ***	3.67 ***
Year	2	417.15 ***	348.36 ***	325.92 ***	10.57 *	1417.25 ***	224.66 ***	38.52 ***	110.50 ***	701.27 ***	2.71 NS	12.81 **
Accession x Year	88	2.01 ***	1.33 *	2.98 ***	1.16 NS	1.78 ***	1.83 ***	2.05 ***	2.66 ***	1.55 *	0.71 NS	0.70 NS

[a] Degrees of freedom. Degrees of freedom for Year and accession x Year for traits LFS, CP, NDF and ADF were 1 and 44 due to no data collection in 2015 for the traits.

[b] ***$P \leq 0.0001$; **$P \leq 0.001$; *$P \leq 0.05$; NS: non-significant.

ADF: acid detergent fiber; CP: crude protein; DH: days to heading; DMY: dry-matter yield; HSV: hay stage vigor; LFS: leafiness; LSR: leaf-to-stem ratio: NDF: neutral detergent fiber; PH: plant height; RG: regrowth; SV: spring vigor.

4.6.1.1 Days to heading

Days to heading was significantly (*P*<0.0001) different among the crested wheatgrass accessions in all three years (Table 4.4). Days to heading ranged from 362 (PI516482) to 475 growing degree days (GDD) (PI401085) in 2015, 332 (PI439914; PI516482; PI439914) to 431 GDD (PI401085) in 2016, and 348 (PI439914; W625134) to 505 GDD (PI401076) in 2017 (Table 4.4). Averaged across the 3 years, the five latest heading accessions were PI401085 (Iran), PI401076 (Iran), S9580 (Canada), PI636511 (Bulgaria) and PI401080 (Iran). Heading date ranged from June 3-17 in 2015, May 26 to June 13 in 2016, and May 29 to June 15 in 2017, respectively (Appendix A). When heading date was compared among the three continents, accessions from North America required significantly (*P*=0.02523) more days to reach heading stage than the accessions from Asia and Europe (Figure 4.2).

Table 4.4 Days to heading of 45 crested wheatgrass accessions in 2015, 2016 and 2017 at Saskatoon SK, Canada

			Days to heading (GDD)		
Accession	Country	Continent	2015	2016	2017
AC Goliath	Canada	North America	393	376	446
AC Parkland	Canada	North America	398	397	451
Kirk	Canada	North America	369	353	380
Newkirk	Canada	North America	367	355	407
S9490	Canada	North America	393	393	461
S9512	Canada	North America	402	377	477
S9516	Canada	North America	412	387	434
S9544	Canada	North America	403	403	446
S9556	Canada	North America	407	403	431
S9571	Canada	North America	403	359	462
S9580	Canada	North America	426	409	489
S9591	Canada	North America	412	403	462
Douglas	USA	North America	383	356	421
PI578519	USA	North America	393	349	420
PI634507	USA	North America	378	336	409
PI636511	Bulgaria	Europe	436	390	482
PI547351	France	Europe	388	377	490
PI281862	Germany	Europe	393	366	472
PI494616	Romania	Europe	383	341	426
PI439914	Russia	Europe	373	332	348
PI547286	Russia	Europe	388	358	404
PI564869	Russia	Europe	384	372	414
PI564879	Russia	Europe	393	369	410
PI564880	Russia	Europe	378	348	383
PI406442	Russia	Europe	378	345	388
PI318922	Spain	Europe	412	377	410
PI297869	Sweden	Europe	417	355	436
PI173622	Turkey	Europe	423	369	457
PI486163	Ukraine	Europe	383	336	431
PI628683	Ukraine	Europe	383	341	402
PI316120	Australia	Australia	369	341	359
PI662330	Armenia	Asia	373	359	426
PI499390	China	Asia	366	332	362
PI547346	China	Asia	457	389	455
PI670374	China	Asia	378	352	384
PI401076	Iran	Asia	431	414	505
PI401080	Iran	Asia	423	408	445
PI401085	Iran	Asia	475	431	495
PI401086	Iran	Asia	393	365	430
PI598641	Kazakhstan	Asia	366	336	358
PI639849	Kazakhstan	Asia	379	351	395
W625134	Kazakhstan	Asia	366	336	348
PI628672	Mongolia	Asia	384	363	382
PI639815	Mongolia	Asia	384	380	415
PI516482	Morocco	Africa	362	332	363
LSD[a] (0.05)			21.2	29.4	49.8
SEM[b]			7.7	10.5	17.8
CV[c] (%)			6.2	7.0	9.8
P value			<0.0001	<0.0001	<0.0001

[a]Least significant difference at P<0.05; [b] Standard error of means; [c] Coefficient of variation.

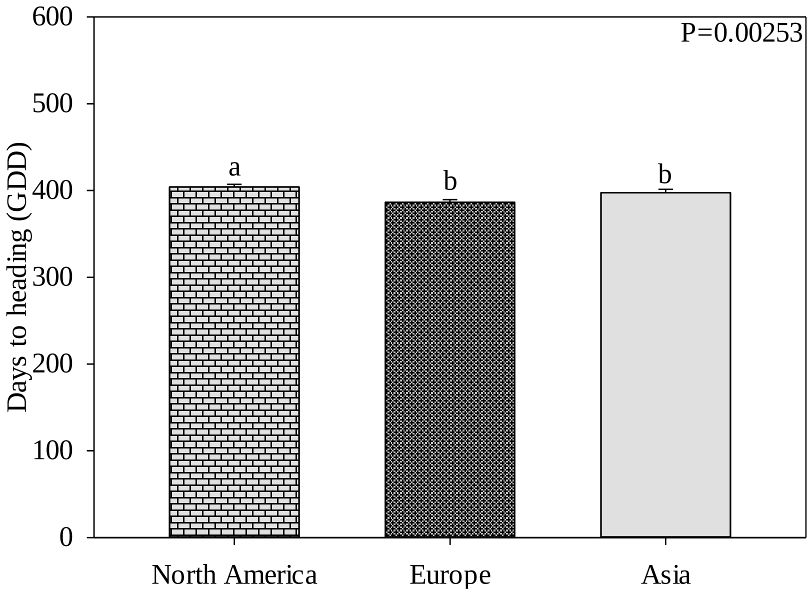


Figure 4.2 Three-year (2015, 2016, and2017) mean days to heading of crested wheatgrass accessions at Saskatoon, SK, Canada expressed by the three continents (Bars represent means ± SE of 4 replicates). Means with different lower-case letters were significantly different ($P \leq 0.05$).

4.6.1.2 Spring vigor and regrowth scores

There was highly significant ($P<0.0001$) difference for spring vigor score among the 45 crested wheatgrass accessions in all three years (Table 4.5). Spring vigor score ranged from 0.9 (PI636511 from Bulgaria) to 3.1 (Newkirk from Canada) in 2015, 1.3 (PI636511 from Mongolia) to 4.6 (PI439914 from Russia) in 2016 and 1.5 (PI401085 from Iran) to 4.1 (S9544, S9556 from Canada; PI634507 from United States; PI486163 from Ukraine) in 2017 (Table 4.5). Averaged across the three years, the five accessions with the highest spring vigor score were PI439914 (Russia), PI486163 (Ukraine), PI634507 (United States), S9544 and S9490 (Canada). Among the three continents, spring vigor varied significantly *($P < 0.0001$)* with accessions from North America having the most vigorous plants compared to the accessions from Europe and Asia (Figure 4.3A).

Table 4.5 Spring vigor and regrowth scores of 45 crested wheatgrass accessions in 2015, 2016 and 2017 at Saskatoon SK, Canada

			Spring vigor[a]			Regrowth[b]		
Accession	Country	Continent	2015	2016	2017	2015	2016	2017
AC Goliath	Canada	North America	2.7	3.7	3.5	2.4	4.1	3.8
AC Parkland	Canada	North America	2.1	4.0	3.8	2.5	3.7	3.1
Kirk	Canada	North America	2.5	4.2	4.0	2.5	3.8	3.7
Newkirk	Canada	North America	3.1	3.9	4.0	2.7	4.1	4.1
S9490	Canada	North America	3.0	3.9	3.8	2.5	4.1	3.8
S9512	Canada	North America	2.7	4.1	3.8	2.6	3.6	3.8
S9516	Canada	North America	2.0	3.4	3.4	1.7	3.4	3.1
S9544	Canada	North America	2.6	4.0	4.1	2.7	4.1	3.8
S9556	Canada	North America	2.3	3.9	4.1	2.2	4.0	4.0
S9571	Canada	North America	2.7	3.8	3.9	2.4	3.9	4.0
S9580	Canada	North America	2.4	3.3	4.0	2.3	3.3	3.7
S9591	Canada	North America	2.3	4.0	3.7	2.3	3.9	3.6
Douglas	USA	North America	2.0	3.0	2.9	1.4	2.0	2.5
PI578519	USA	North America	2.4	3.8	4.0	2.4	3.8	3.6
PI634507	USA	North America	2.1	4.5	4.1	2.2	4.1	3.5
PI636511	Bulgaria	Europe	0.9	2.0	2.3	1.3	2.3	2.2
PI547351	France	Europe	1.1	2.2	2.9	1.2	2.6	2.6
PI281862	Germany	Europe	1.9	3.8	3.9	2.1	3.8	2.6
PI494616	Romania	Europe	1.3	3.1	3.1	1.3	3.0	2.9
PI439914	Russia	Europe	2.4	4.6	4.0	2.4	4.0	3.4
PI547286	Russia	Europe	1.1	2.7	2.2	1.3	1.9	2.9
PI564869	Russia	Europe	1.3	2.1	2.6	1.2	2.4	2.6
PI564879	Russia	Europe	1.2	2.0	2.4	1.2	2.1	2.1
PI564880	Russia	Europe	1.5	2.5	2.8	1.4	2.5	2.7
PI406442	Russia	Europe	1.7	3.7	2.8	1.3	2.7	2.7
PI318922	Spain	Europe	1.2	1.4	2.1	1.2	2.1	2.5
PI297869	Sweden	Europe	1.1	1.5	2.0	1.1	1.6	2.0
PI173622	Turkey	Europe	1.5	2.9	2.5	1.4	1.9	2.5
PI486163	Ukraine	Europe	2.3	4.5	4.1	2.3	3.6	3.8
PI628683	Ukraine	Europe	1.1	2.0	2.7	1.1	2.1	2.3
PI316120	Australia	Australia	2.4	4.1	3.7	1.9	2.9	2.9
PI662330	Armenia	Asia	1.3	2.1	2.4	1.2	2.1	2.2
PI499390	China	Asia	1.4	2.6	3.3	1.6	2.8	3.3
PI547346	China	Asia	1.1	2.0	1.6	1.0	1.3	1.9
PI670374	China	Asia	1.3	1.6	2.0	1.4	1.8	2.7
PI401076	Iran	Asia	1.2	2.1	1.6	1.3	2.0	1.9
PI401080	Iran	Asia	1.1	2.0	1.9	1.1	1.8	2.5
PI401085	Iran	Asia	1.0	1.4	1.5	1.0	1.4	1.9
PI401086	Iran	Asia	1.0	2.1	1.9	1.1	1.3	1.8
PI598641	Kazakhstan	Asia	1.9	3.1	3.8	2.0	3.5	3.4
PI639849	Kazakhstan	Asia	2.2	3.3	3.4	1.7	2.9	3.1
W625134	Kazakhstan	Asia	2.1	3.5	3.1	1.8	3.6	3.2
PI628672	Mongolia	Asia	0.9	1.4	2.2	1.1	2.0	2.1
PI639815	Mongolia	Asia	1.0	1.3	1.6	1.1	1.7	1.8
PI516482	Morocco	Africa	2.0	3.8	3.3	2.0	3.7	3.4
LSD[c] (0.05)			0.6	0.7	0.6	0.5	0.8	0.7
SEM[d]			0.2	0.3	0.2	0.2	0.3	0.2
CV[c] (%)			35.6	33.6	28.1	33.1	32.6	28.1
P-value			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a]Spring vigor score: 1-5 (1 = poor, 5 = good); [b]Regrowth score: 1-5 (1 = slow, 5 = rapid), [c]Least significant difference at $P \leq 0.05$; [d]Standard error of mean; [c] Coefficient of variation.

Regrowth score varied (*P*<0.0001) among the 45 crested wheatgrass accessions during the three years of study (Table 4.5). Regrowth score ranged from 1.0 (PI401085 from Iran) to 2.7 (Newkirk and S9544 from Canada) in 2015, 1.2 (PI401086 from Iran) to 4.6 (S9490, S9544, Newkirk, AC Goliath from Canada; and PI634507 from United States) in 2016 and 1.8 (PI401085 from Iran, and PI639815 from Mongolia) to 4.1 (Newkirk from Canada) in 2017 (Table 4.5). Accessions from North America had significantly *(P <0.0001)* higher regrowth score than the accessions from Europe and Asia (Figure 4.3B). Canadian cultivars Newkirk, AC Goliath and breeding lines S9544, S9490, S9571, and S9556 were the six accessions with the highest regrowth score.

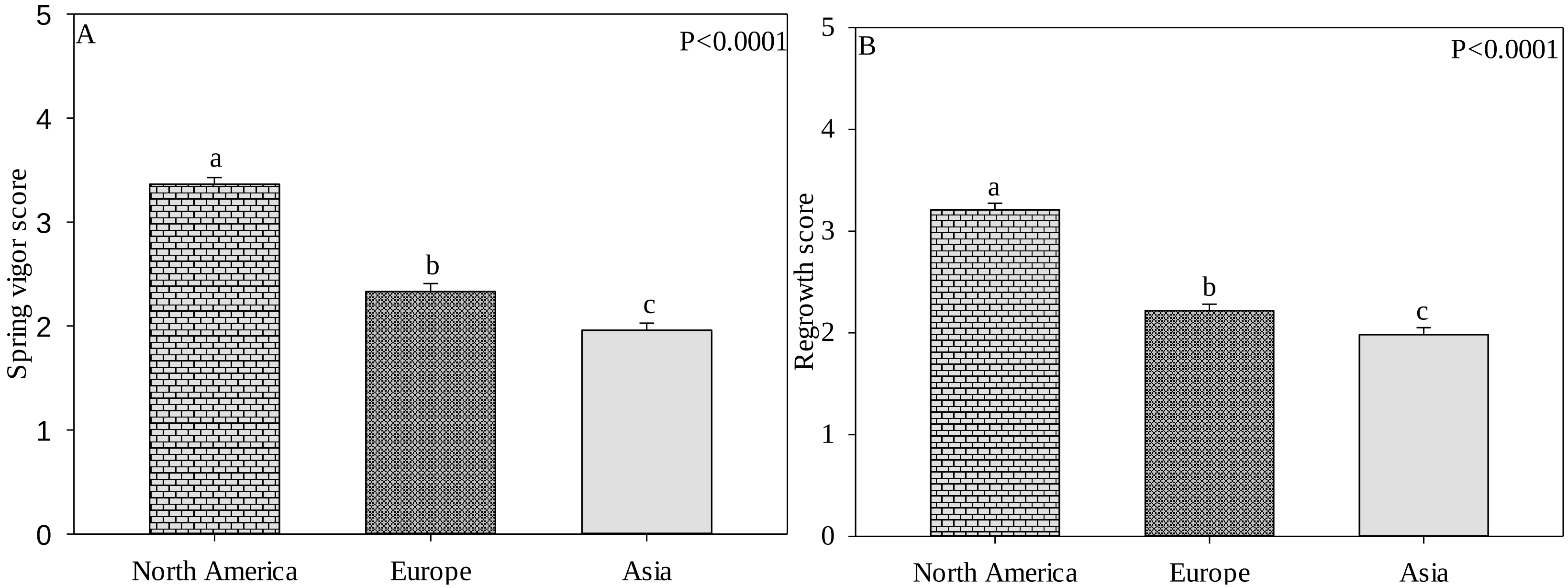


Figure 4.3 Three-year (2015, 2016 and 2017) mean of A) spring vigor score and B) regrowth score for crested wheatgrass accessions expressed by continent of origin at Saskatoon SK Canada (Bars represent means ± SE of 4 replicates). Means with different lower-case letters were significantly different ($P \leq 0.05$).

4.6.1.3 Leaf-to-stem ratio and dry matter (DM) yield

Leaf-to-stem ratio was significantly different among accessions (*P*<0.0001), years (*P*<0.0001), and their interaction (*P*<0.0001) (Table 4.3). In 2015, leaf-to-stem ratio for 45 crested wheatgrass accessions ranged from 0.10 (Douglas from United States, W625134 from Kazakhstan) to 0.25 (PI499390 form China) in 2015; from 0.10 (PI670374 from China) to 0.27 (PI 401080 from Iran) in 2016; and from 0.14 (PI639815 from Mongolia, PI547346 from China) to 0.35 (AC Parkland from Canada) in 2017, respectively (Table 4.6). Averaged across the three years, accessions with the highest leaf-to-stem ratio were PI486163 (Ukraine), AC Parkland, S9591, S9544 (all from Canada) and PI401080 (Iran). In addition, leaf-to-stem ratio was significantly (*P* < 0.0001) higher in North American accessions than in European and Asian accessions (Figure 4.4A). In addition to the leaf-to-stem ratio measurement, visual scores of leafiness was recorded for individual plants (Appendix B). Similar to the leaf-to-stem ratio results, leafiness score varied significantly (*P*<0.0001) among the 45 crested wheatgrass accessions and among the three continents (Appendix C).

Table 4.6 Leaf-to-stem ratio and dry matter yield of 45 crested wheatgrass accessions in 2015, 2016 and 2017 at Saskatoon SK, Canada

			Leaf-to-stem ratio			Dry matter yield (g plant^{-1})		
Accession	Country	Continent	2015	2016	2017	2015	2016	2017
AC Goliath	Canada	North America	0.13	0.18	0.25	63	212	289
AC Parkland	Canada	North America	0.13	0.25	0.35	56	228	271
Kirk	Canada	North America	0.14	0.18	0.25	102	243	275
Newkirk	Canada	North America	0.11	0.19	0.24	48	209	270
S9490	Canada	North America	0.16	0.20	0.22	35	197	299
S9512	Canada	North America	0.14	0.22	0.25	106	209	278
S9516	Canada	North America	0.13	0.23	0.26	46	186	232
S9544	Canada	North America	0.14	0.26	0.26	76	253	315
S9556	Canada	North America	0.15	0.24	0.25	32	262	310
S9571	Canada	North America	0.15	0.20	0.26	74	228	316
S9580	Canada	North America	0.14	0.23	0.26	48	201	241
S9591	Canada	North America	0.13	0.24	0.28	60	239	293
Douglas	USA	North America	0.10	0.22	0.26	59	150	146
PI578519	USA	North America	0.14	0.21	0.22	49	231	285
PI634507	USA	North America	0.14	0.21	0.25	61	198	237
PI636511	Bulgaria	Europe	0.13	0.18	0.23	56	75	68
PI547351	France	Europe	0.17	0.22	0.20	49	141	132
PI281862	Germany	Europe	0.13	0.21	0.26	112	204	198
PI494616	Romania	Europe	0.17	0.16	0.23	81	133	162
PI439914	Russia	Europe	0.19	0.16	0.20	82	221	232
PI547286	Russia	Europe	0.15	0.23	0.19	53	111	105
PI564869	Russia	Europe	0.14	0.15	0.16	62	116	128
PI564879	Russia	Europe	0.12	0.14	0.17	66	116	131
PI564880	Russia	Europe	0.12	0.14	0.16	77	89	151
PI406442	Russia	Europe	0.17	0.22	0.25	109	149	131
PI318922	Spain	Europe	0.15	0.24	0.22	56	63	114
PI297869	Sweden	Europe	0.13	0.16	0.19	79	45	76
PI173622	Turkey	Europe	0.13	0.23	0.23	123	127	112
PI486163	Ukraine	Europe	0.21	0.23	0.34	88	263	325
PI628683	Ukraine	Europe	0.15	0.17	0.18	45	71	153
PI316120	Australia	Australia	0.13	0.19	0.17	90	162	189
PI662330	Armenia	Asia	0.25	0.12	0.18	43	80	118
PI499390	China	Asia	0.19	0.14	0.14	78	123	169
PI547346	China	Asia	0.13	0.19	0.18	55	89	75
PI670374	China	Asia	0.13	0.10	0.16	83	82	146
PI401076	Iran	Asia	0.14	0.25	0.24	65	118	71
PI401080	Iran	Asia	0.23	0.27	0.16	49	114	105
PI401085	Iran	Asia	0.15	0.26	0.19	51	87	66
PI401086	Iran	Asia	0.18	0.20	0.23	97	69	73
PI598641	Kazakhstan	Asia	0.15	0.16	0.15	77	200	217
PI639849	Kazakhstan	Asia	0.12	0.23	0.23	49	131	192
W625134	Kazakhstan	Asia	0.10	0.15	0.18	50	129	215
PI628672	Mongolia	Asia	0.14	0.12	0.14	43	35	83
PI639815	Mongolia	Asia	0.13	0.14	0.14	88	56	77
PI516482	Morocco	Africa	0.17	0.17	0.16	43	212	195
LSD[a] (0.05)			0.06	0.06	0.07	70	60	65
SEM[b]			0.02	0.02	0.03	25.1	22.1	24.2
CV[c]			20.4	22.3	23.0	33.0	43.5	45.2
P value			0.0005	<0.0001	<0.0001	0.859	<0.0001	<0.0001

[a] Least significant difference at $P \leq 0.05$; [b] Standard error of mean; [c] Coefficient of variation.

Dry matter yield was significantly ($P<0.0001$) different among the accessions. Year and accession x year interaction were also significant for DM yield (Table 4.3). In 2015, DM yield of 45 accessions ranged from 32 g plant^{-1} (S9556 from Canada) to 123 g plant^{-1} (PI173622 from Turkey); 35g plant^{-1} (PI628672 from Mongolia) to 263 g plant^{-1} (PI486163 from Ukraine) in 2016; and from 66 g plant^{-1} (PI401085 from Iran) to 325 g plant^{-1} (PI486163 from Ukraine) in 2017. (Table 4.6). Averaged across the three years, the five accessions with the highest DM yield were PI486163 (Ukraine), Canadian cultivar Kirk, and breeding lines S9544, S9556, S9571 (Canada). Dry matter yield significantly ($P<0.0001$) varied among the three continents with North American accessions having the highest average DM yield of 183 g plant^{-1} compared to European (114 g plant^{-1}) and Asian (96 g plant^{-1}) accessions (Figure 4.4B). In addition, individual plant vigor was scored at the early anthesis stage, which is a recommended hay harvest stage (Appendix B). Therefore, it was called hay stage vigor. Similar to forage DM yield, there were highly significant ($P<0.0001$) differences among the 45 crested wheatgrass accessions for hay stage vigor score across all years and among the three continent (Appendix B, C).

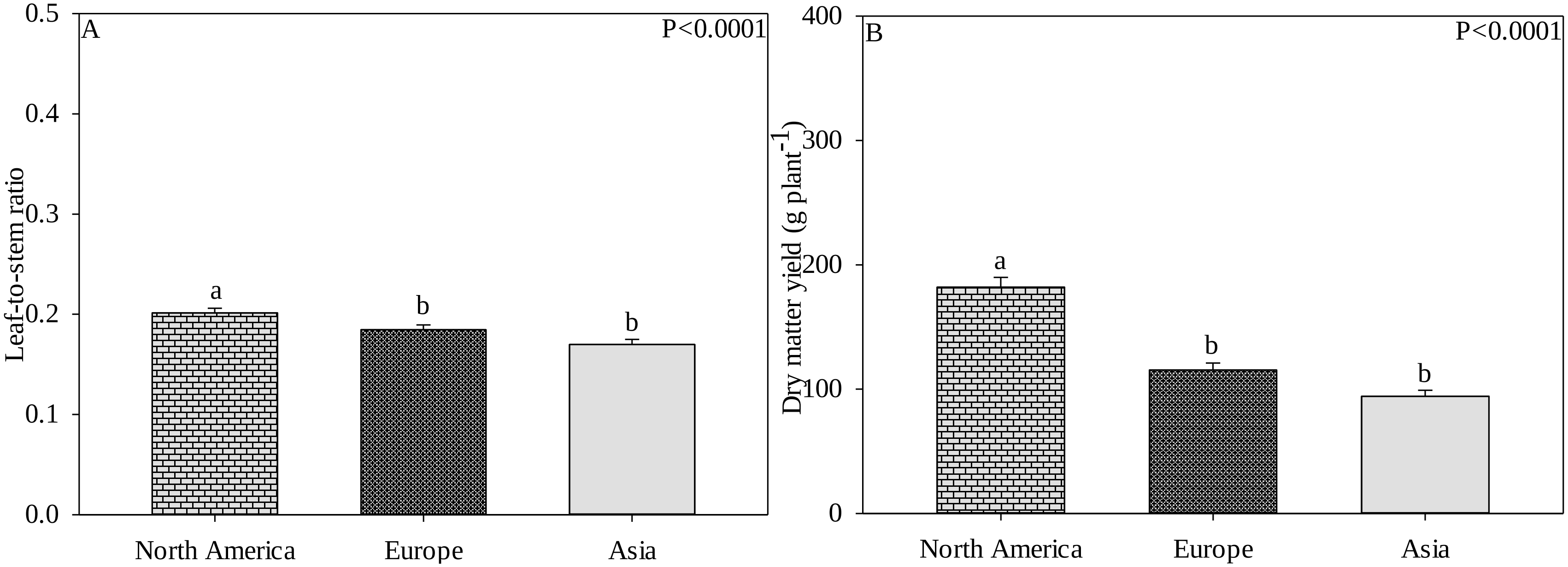


Figure 4.4 Three-year mean (2015, 2016 and 2017) of A) leaf-to-stem ratio and B) forage DM yield (g plant^{-1}) of 45 crested wheatgrass accessions expressed by continent of origin at Saskatoon SK Canada (Bars represent means ± SE of 4 replicates). Means with different lower-case letters were significantly different (*P*≤0.05).

4.6.1.4 Plant height

Plant height varied significantly ($P<0.001$) among crested wheatgrass accessions. Year and accession x year interaction were also significant ($P<0.001$) for plant height (Table 4.3). Plant height ranged from 31 cm (PI636511 from Bulgaria) to 78 cm (AC Goliath from Canada) in 2015, from 40 cm (PI547351 from France) to 80 cm (S9556 from Canada) in 2016, and from 43cm (PI639815 from Mongolia) to 83 cm (S9571 from Canada) in 2017 (Table 4.7). Cultivar AC Goliath, and breeding lines S9571, S9556, S95544 and S9490 (all from Canada) were the tallest accessions. In addition, crested wheatgrass plants in North American accessions were significantly taller than those in European and Asian accessions (Figure 4.5).

Table 4.7 Plant height of 45 crested wheatgrass accessions in 2015, 2016 and 2017 at Saskatoon SK, Canada

			Plant height (cm)		
Accession	Country	Continent	2015	2016	2017
AC Goliath	Canada	North America	78	78	79
AC Parkland	Canada	North America	64	68	70
Kirk	Canada	North America	75	75	70
Newkirk	Canada	North America	75	75	78
S9490	Canada	North America	76	74	79
S9512	Canada	North America	64	71	76
S9516	Canada	North America	65	74	74
S9544	Canada	North America	75	77	79
S9556	Canada	North America	74	80	78
S9571	Canada	North America	77	79	83
S9580	Canada	North America	68	76	83
S9591	Canada	North America	70	76	75
Douglas	USA	North America	63	66	66
PI578519	USA	North America	61	67	68
PI634507	USA	North America	58	67	64
PI636511	Bulgaria	Europe	31	56	61
PI547351	France	Europe	41	40	46
PI281862	Germany	Europe	47	61	61
PI494616	Romania	Europe	41	52	55
PI439914	Russia	Europe	74	79	66
PI547286	Russia	Europe	50	59	58
PI564869	Russia	Europe	53	55	53
PI564879	Russia	Europe	51	52	53
PI564880	Russia	Europe	54	57	58
PI406442	Russia	Europe	66	75	64
PI318922	Spain	Europe	47	47	57
PI297869	Sweden	Europe	37	44	51
PI173622	Turkey	Europe	50	60	60
PI486163	Ukraine	Europe	63	72	70
PI628683	Ukraine	Europe	43	48	55
PI316120	Australia	Australia	76	73	64
PI662330	Armenia	Asia	53	54	55
PI499390	China	Asia	63	60	59
PI547346	China	Asia	49	58	57
PI670374	China	Asia	59	54	55
PI401076	Iran	Asia	50	59	58
PI401080	Iran	Asia	55	59	61
PI401085	Iran	Asia	51	58	52
PI401086	Iran	Asia	48	53	50
PI598641	Kazakhstan	Asia	63	67	64
PI639849	Kazakhstan	Asia	59	61	62
W625134	Kazakhstan	Asia	67	66	62
PI628672	Mongolia	Asia	47	51	55
PI639815	Mongolia	Asia	46	45	43
PI516482	Morocco	Africa	61	68	58
LSD[a] (0.05)			8.7	7.1	7.7
SEM[b]			3.2	2.6	2.8
CV[c]			20.6	17.1	16.0
P value			<0.0001	<0.0001	<0.0001

[a]Least significant difference at $P \leq 0.05$; [b]Standard error of mean; [c] Coefficient of variation.

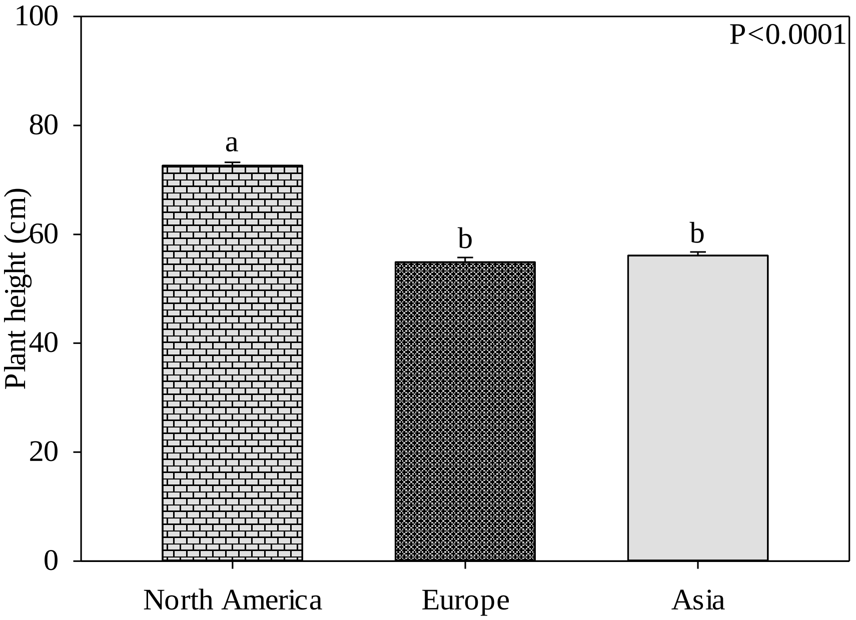


Figure 4.5 Three-year (2015, 2016 and 2017) mean plant height (cm) for 45 crested wheatgrass accessions expressed by continent of origin at Saskatoon SK Canada (Bars represent means ± SE of 4 replicates). Means with different lower-case letters were significantly different ($P \leq 0.05$).

4.6.1.5 Forage nutritive value

Accessions ($P<0.0001$), year ($P<0.0001$) and their interaction ($P<0.05$) had significant effects on crude protein concentration of crested wheatgrass (Table 4.3). Concentrations of CP ranged from 104 g kg^{-1} DM (PI547346 from China) to 139 g kg^{-1} DM (PI547351 from France) (Table 4.8). Crested wheatgrass accessions with the highest CP concentrations were PI547351 (France), PI628672 (Mongolia), PI564869 (Russia) and PI639815 (Mongolia). When grouped by continent of origin, CP concentrations were not different among the three continents (Figure 4.6).

Year and accession had significant ($P<0.001$; $P<0.0001$) effects on ADF and NDF concentrations of crested wheatgrass respectively, but accession x year interactions were not significant for NDF ($P=0.91$) and ADF ($P=0.93$) (Table 4.3). Neutral detergent fiber concentration ranged from 546 g kg^{-1} DM (PI439914 from Russia) to 618 g kg^{-1} DM (PI639815 from Mongolia) (Table 4.8).

Table 4.8 Average concentrations (g kg^{-1} DM) of crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) of 45 crested wheatgrass accessions in 2016 and 2017 at Saskatoon SK, Canada

Accession	Country	Continent	CP	NDF	ADF
AC Goliath	Canada	North America	120	608	371
AC Parkland	Canada	North America	127	579	334
Kirk	Canada	North America	117	600	358
Newkirk	Canada	North America	118	608	363
S9490	Canada	North America	114	599	375
S9512	Canada	North America	110	562	362
S9516	Canada	North America	126	600	322
S9544	Canada	North America	123	609	363
S9556	Canada	North America	124	595	363
S9571	Canada	North America	115	600	363
S9580	Canada	North America	121	607	358
S9591	Canada	North America	125	605	357
Douglas	USA	North America	123	579	353
PI578519	USA	North America	120	604	366
PI634507	USA	North America	125	605	357
PI636511	Bulgaria	Europe	126	591	340
PI547351	France	Europe	139	583	343
PI281862	Germany	Europe	118	557	313
PI494616	Romania	Europe	125	580	337
PI439914	Russia	Europe	105	546	329
PI547286	Russia	Europe	120	586	345
PI564869	Russia	Europe	130	593	332
PI564879	Russia	Europe	117	617	366
PI564880	Russia	Europe	118	581	331
PI406442	Russia	Europe	112	553	333
PI318922	Spain	Europe	126	614	372
PI297869	Sweden	Europe	127	588	339
PI173622	Turkey	Europe	121	596	350
PI486163	Ukraine	Europe	122	587	347
PI628683	Ukraine	Europe	126	589	354
PI316120	Australia	Australia	113	586	352
PI662330	Armenia	Asia	125	592	364
PI499390	China	Asia	114	596	356
PI547346	China	Asia	104	594	351
PI670374	China	Asia	107	613	368
PI401076	Iran	Asia	125	584	349
PI401080	Iran	Asia	122	589	357
PI401085	Iran	Asia	127	582	347
PI401086	Iran	Asia	121	585	360
PI598641	Kazakhstan	Asia	113	586	352
PI639849	Kazakhstan	Asia	128	618	358
W625134	Kazakhstan	Asia	123	594	366
PI628672	Mongolia	Asia	130	611	371
PI639815	Mongolia	Asia	128	618	358
PI516482	Africa	Africa	123	586	336
LSD[a]			12.5	23.5	20.7
SEM[b]			8.39	17.67	8.39
P-value			<0.0001	<0.0001	<0.0001

[a]Least significant difference at P≤ 0.05, [b]Standard error of means.
Values represent mean of two years data.

The concentration of ADF ranged from 313 g kg^{-1} DM (PI281862 from Germany) to 375 g kg^{-1} DM (Newkirk from Canada) (Table 4.8). Among the three continents, ADF, and NDF concentrations varied significantly *(P = 0.0016)* with European accessions having significantly lower concentration compared to accessions from North America and Asia (Figure 4.6B, C). PI439914 (Russia), PI281862 (Germany) and PI4046442 (Russia) and AC Parkland (Canada) were the accessions with the lowest mean ADF and NDF concentrations.

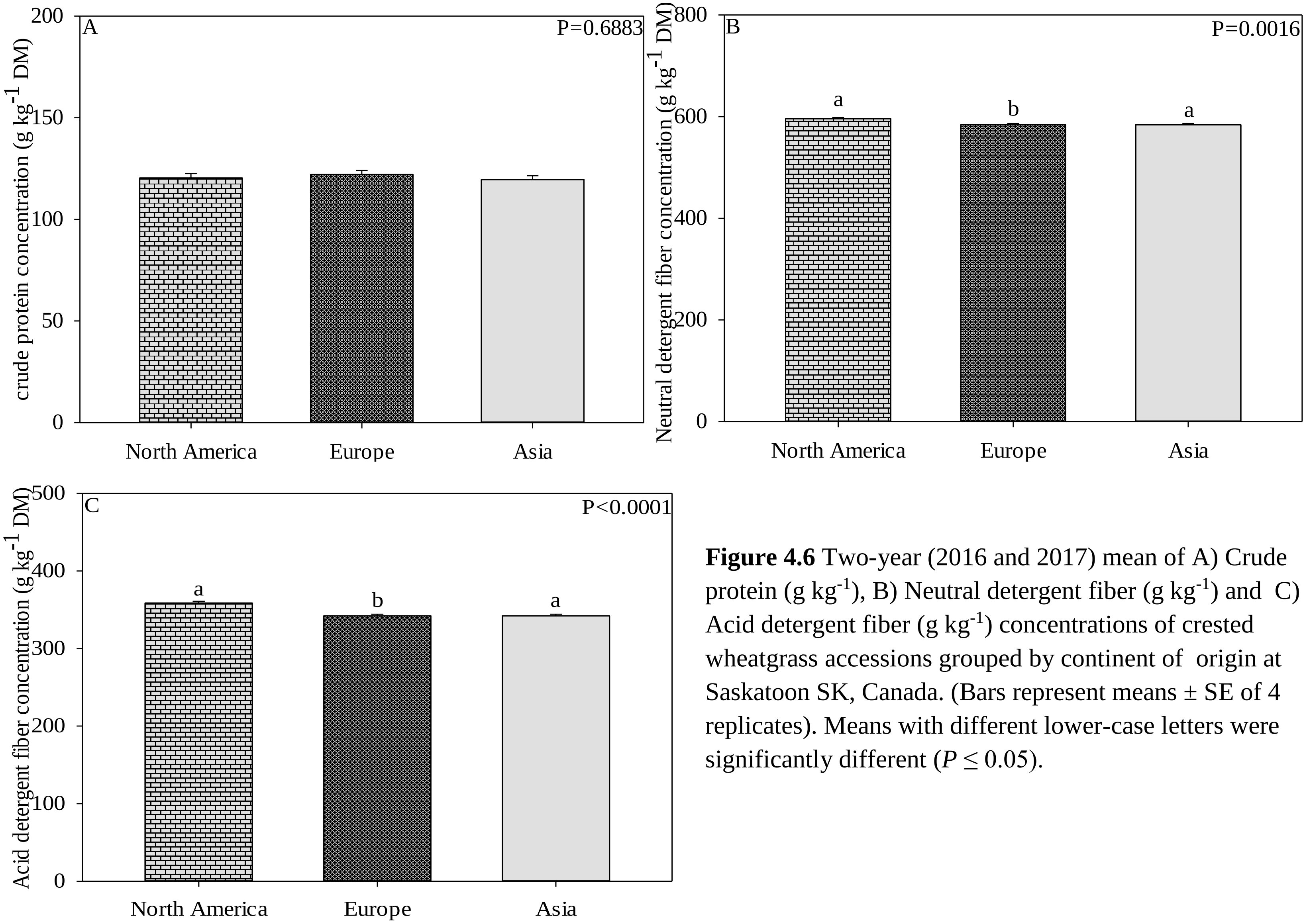


Figure 4.6 Two-year (2016 and 2017) mean of A) Crude protein (g kg^{-1}), B) Neutral detergent fiber (g kg^{-1}) and C) Acid detergent fiber (g kg^{-1}) concentrations of crested wheatgrass accessions grouped by continent of origin at Saskatoon SK, Canada. (Bars represent means ± SE of 4 replicates). Means with different lower-case letters were significantly different ($P \leq 0.05$).

4.6.1.6 Association among agro-morphological traits and nutritive value

Coefficients of correlation between any two measured traits are shown in Table 4.9. Days to heading of crested wheatgrass showed positive correlations with leaf-to-stem ratio (r=0.23, $P<0.0001$), NDF (r=0.16, $P<0.05$), ADF (r=0.18, $P<0.001$), but no significant relationship was found between days to heading and forage DM yield (r= 0.07, $P=0.09$), plant height (r=0.002, $P=0.96$) and regrowth (r=-0.06, $P=0.17$). Late maturing accessions had low CP (r=-0.30, $P<0.0001$) and low spring vigor score (r=-0.11, $P<0.05$) (Table 4. 9). Forage DM yield had a positive correlation with spring vigor (r=0.77, $P<0.0001$), leaf-to-stem ratio (r=0.50, $P<0.0001$), plant height (r=-0.56, $P<0.0001$), regrowth (r=0.67, $P<0.0001$), hay stage vigor (r=0.58, $P<0.0001$), leafiness (r=0.79, $P<0.0001$), and ADF (r=0.11, $P<0.05$) (Table 4.9). There was a negative correlation between forage DM yield and CP concentration (r=-0.23, $P<0.001$), but no significant relationship was found between DM yield and NDF (r=0.04, $P=0.46$) (Table 4.9). Crude protein showed negative correlation with spring vigor (r=-0.14, $P<0.05$), hay stage vigor (r=−0.37, $P<0.0001$), leafiness (r=−0.15, $P<0.05$), NDF (r=−0.18, $P<0.05$) and ADF (r=−0.18, $P<0.001$) (Table 4.9). Furthermore, ADF had significant positive correlations with plant height (r=0.64, $P<0.0001$) and NDF (r=0.64, $P<0.0001$) (Table 4.9).

Data were further analyzed for adapted accessions from Canada, and the majority of results were similar to the results of correlation analysis for all the 45 crested wheatgrass accessions (Table 4.9). Days to heading was positively correlated to plant height (r= 0.23, $P<0.05$), DM yield (r= 0.34, $P<0.0001$), hay stage vigor (r= 0.47, $P<0.0001$), leafiness (r= 0.24, $P<0.05$) (Table 4.10). Days to heading showed no significant relationship NDF (r= 0.12, $P=0.26$), ADF (r= 0.09, $P=0.40$) and spring vigor (r= 0.06, $P=0.44$) (Table 4.10). DM yield showed a non-significant correlation with ADF (r= 0.12, $P=0.23$) (Table 4.10). However, crude protein showed negative correlation with leaf-to-stem ratio (r=-0.32, $P<0.05$) and NDF (r=-0.32, $P<0.05$) but a non-significant correlation with spring vigor (r=-0.02, $P=0.82$).

Table 4.9 Coefficient of correlation (r) among 11 traits measured on 45 crested wheatgrass accessions evaluated in 2015, 2016 and 2017 at Saskatoon SK, Canada

	SV[a]	DMY	LSR	DH	PH	RG	HSV	LFS	CP	NDF	ADF
SV	1[b]										
DMY	0.77***	1									
LSR	0.37***	0.50***	1								
DH	-0.11*	NS	0.23***	1							
PH	0.72***	0.56***	0.27***	NS	1						
RG	0.62***	0.67***	0.33***	NS	0.62***	1					
HSV	0.66***	0.58***	0.33***	NS	0.44***	0.55***	1				
LFS	0.77***	0.79***	0.39***	NS	0.66***	0.56***	0.46***	1			
CP	-0.14*	-0.23***	NS	-0.31***	NS	NS	-0.37***	-0.15*	1		
NDF	NS	NS	NS	0.16*	NS	NS	NS	NS	-0.13*	1	
ADF	NS	0.11*	NS	0.18**	0.19**	NS	NS	NS	-0.18**	0.64***	1

[a]ADF: acid detergent fiber concentration; CP: crude protein concentration; DH: days to heading; DMY: dry matter yield; HSV: hay stage vigor; LFS: leafiness; LSR: leaf-to-stem ratio; NDF: neutral detergent fiber concentration; PH: plant height; RG: regrowth; SV: spring vigor.

[b]Coefficient of correlation (r); [c]***, significant at P<0.0001; **, significant at P <0.001; *, significant at P <0.05; NS, non-significant

Table 4.10 Coefficient of correlation (r) among 11 traits measured on 12 Canadian crested wheatgrass accessions evaluated in 2015, 2016 and 2017 at Saskatoon SK, Canada

	SV[a]	DMY	LSR	DH	PH	RG	HSV	LFS	CP	NDF	ADF
SV	1[b]										
DMY	0.75***	1									
LSR	0.57***	0.74***	1								
DH	NS	0.34***	0.40***	1							
PH	0.51***	0.44***	0.23*	0.23*	1						
RG	0.72***	0.68***	0.55***	NS	0.41***	1					
HSV	0.48***	0.56***	0.49***	0.47***	0.34***	0.33***	1				
LFS	0.47***	0.51***	0.27*	0.24*	0.39***	0.56***	NS	1			
CP	NS	-0.44***	-0.32*	-0.50***	NS	NS	-0.65***	-0.23*	1		
NDF	NS	NS	NS	NS	NS	NS	NS	NS	NS	1	
ADF	NS	NS	NS	NS	0.31*	NS	NS	NS	-0.23*	0.54***	1

[a]ADF: acid detergent fiber concentration; CP: crude protein concentration; DH: days to heading; DMY: dry matter yield; HSV: hay stage vigor; LFS: leafiness; LSR: leaf-to-stem ratio; NDF: neutral detergent fiber concentration; PH: plant height; RG: regrowth; SV: spring vigor.

[b]Coefficient of correlation (r); [c]***, significant at P<0.0001; **, significant at P <0.001; *, significant at P <0.05; NS, non-significant

4.6.1.7 Within-accession variation

Variation within an accession was calculated for all the measured traits (Table 4.11). The magnitude of variation for days to heading was the greatest in accessions PI547351 (France), S9580 (Canada), PI281862 (Germany), S9512 (Canada) and PI173622 (Turkey) (Table 4.11). Variability for plant height was high in accessions PI636511 (Bulgaria), PI639849 (Kazakhstan), S9516, S9580 and S9591 (Canada) (Table 4.11). Variation range for DM yield was high in accessions S9556, S9591, S9544, S9590 and Newkirk (from Canada) (Table 4.11). Accessions with numerically the highest variability for leaf-to-stem ratio were PI318922 (Spain), PI401080 (Iran), S9591 and AC Parkland (Canada) and PI499390 (China) (Table 4.11). High variation for spring vigor score was detected in accessions PI634507 (United States), PI486163 (Ukraine), PI281862 (Germany), S9591 and AC Parkland (Canada) (Table 4.11). Accessions PI634507 (United States), S9580, S9591, S9516 and S9556 (from Canada) had the highest variability for regrowth score (Table 4.11).

The highest within-accession variability for crude protein was observed in accessions AC Goliath, S9490, S9571, S9591 and S9516 (all from Canada) (Table 4.11). Accessions S9571 and S9556 (Canada), PI639815 (Mongolia), PI662330 (Armenia) and PI316120 (Australia) showed the highest variability in terms of neutral detergent fiber concentration (Table 4.11). Within-accession variation for acid detergent fiber the highest variability in accessions S9591, AC Goliath (both from Canada), PI494616 (Romania), PI639849 (Kazakhstan) and (PI316120 (Australia) (Table 4.11).

Table 4.11 Means and standard deviation for 11 agronomic and morphological characters of 45 crested wheatgrass accessions in 2015, 2016 &2017 at Saskatoon SK, Canada

Accession	Days to heading (GDD	Spring vigor (1-5)	Regrowth (1-5)	Leafiness (1-5)	Hay stage vigor (1-5)	Plant Height (cm)	Dry matter yield (g plant^{-1})	Leaf-to-stem ratio	CP (g kg^{-1})	NDF (g kg^{-1})	ADF (g kg^{-1})
AC Goliath	405±38	3.3±0.5	3.4±0.8	4.1±0.6	3.3±0.8	78±3.5	188±106	0.10±0.07	120±38	608±27	371±37
AC Parkland	415±40	3.3±1.0	3.1±0.6	4.5±0.2	3.4±1.1	67±5.3	185±107	0.24±0.10	127±26	579±22	334±21
Kirk	367±18	3.6±0.9	3.3±0.7	4.1±0.5	3.1±1.0	73±4.5	206±99	0.18±0.05	117±20	600±11	363±11
Newkirk	376±31	3.7±0.8	3.6±0.9	3.8±1.1	3.1±1.0	76±7.3	175±116	0.18±0.06	119±27	608±19	375±22
S9490	416±39	3.6±0.7	3.5±0.7	3.9±1.2	3.1±0.9	77±4.0	177±118	0.19±0.04	126±31	600±15	363±13
S9512	419±48	3.5±0.7	3.3±0.8	3.8±1.0	3.2±0.9	70±6.4	198±84.	0.20±0.06	123±21	609±19	363±24
S9516	411±45	3.0±0.8	2.8±1.0	3.8±0.6	2.8±1.2	72±12.0	175±107	0.21±0.07	124±28	595±19	363±25
S9544	417±26	3.6±0.9	3.5±0.8	4.6±0.3	3.2±1.2	77±3.5	214±118	0.22±0.07	115±25	600±20	358±18
S9556	414±20	3.4±0.9	3.4±1.0	4.5±0.4	3.2±1.1	77±5.5	217±130	0.21±0.05	121±25	607±36	371±24
S9571	408±46	3.4±0.6	3.4±0.8	4.3±0.6	3.4±1.0	80±5.0	206±110	0.20±0.06	123±30	579±47	353±18
S9580	441±54	3.3±0.9	3.1±1.0	4.2±0.9	3.1±0.9	76±9.3	163±103	0.21±0.07	120±27	604±22	366±25
S9591	426±30	3.2±1.0	3.1±1.0	4.2±0.3	2.9±1.1	72±8.9	173±120	0.20±0.08	125±29	605±22	357±39
Douglas	386±34	2.6±0.6	2.0±0.6	3.3±0.5	2.6±0.9	65±4.6	118±56	0.19±0.07	123±21	584±12	358±12
PI173622	416±47	2.3±0.9	1.9±0.7	2.8±0.9	2.4±1.0	57±6.3	121±28	0.20±0.07	121±14	596±18	350±20
PI281862	410±49	3.2±1.1	2.8±0.8	4.3±0.6	3.1±1.3	56±7.3	172±65	0.20±0.06	118±25	557±24	313±18
PI297869	403±40	1.5±0.5	1.5±0.5	2.0±0.9	2.2±1.1	44±7.7	66±38	0.16±0.05	127±19	588±22	339±15
PI316120	356±14	3.4±0.9	2.6±0.6	3.7±0.6	2.8±0.8	71±6.3	147±61	0.16±0.04	113±18	586±33	352±29
PI318922	400±32	1.6±0.8	1.9±0.9	2.0±1.0	2.5±1.2	50±9.2	79±77	0.20±0.11	126±27	614±9	372±17
PI401076	450±42	1.6±0.5	1.7±0.9	2.7±0.7	2.3±0.8	56±5.3	85±41	0.21±0.07	125±15	584±25	349±24
PI401080	425±38	1.7±0.5	1.8±0.7	2.9±0.6	2.3±1.0	58±4.3	93±49	0.22±0.09	122±21	589±19	357±19
PI401085	467±35	1.3±0.4	1.4±0.5	2.8±0.7	2.0±0.8	54±5.3	68±49	0.20±0.07	127±20	582±23	347±25
PI401086	396±49	1.7±0.6	1.4±0.5	2.4±0.7	2.3±1.2	50±5.5	80±36	0.20±0.04	121±14	585±18	360±11
PI406442	370±22	2.7±1.0	2.2±0.7	2.6±0.6	2.7±1.2	68±7.5	130±52	0.21±0.06	112±20	553±14	333±16
PI439914	351±18	3.7±1.0	3.3±0.7	3.9±0.6	3.6±1.1	73±6.3	178±76	0.18±0.06	102±16	546±24	329±10
PI486163	383±45	3.6±1.1	3.2±0.8	4.7±0.3	3.6±1.2	68±5.7	226±113	0.26±0.07	122±21	587±12	347±21
PI494616	383±45	2.5±1.0	2.4±1.0	3.7±1.0	2.9±1.4	49±7.4	130±53	0.19±0.06	125±22	580±27	337±35
PI499390	386±45	1.9±0.6	1.8±0.6	1.9±0.7	2.3±1.0	54±4.4	80±47	0.18±0.08	114±22	596±24	356±14
PI516482	352±16	3.0±1.0	3.1±0.8	3.3±0.7	3.0±1.0	62±5.7	150±88	0.16±0.04	123±26	586±29	336±18
PI547286	383±30	2.0±0.8	2.0±0.8	2.9±1.1	2.5±1.4	55±7.8	90±7	0.19±0.07	120±19	586±21	345±19
PI547346	353±18	2.4±0.9	2.6±0.8	3.0±0.3	3.0±1.1	61±4.8	123±49	0.15±0.05	104±7	594±28	351±29
PI547351	418±59	2.1±0.8	2.1±0.8	3.5±0.7	3.0±1.2	42±4.8	107±61	0.20±0.05	139±28	583±12	343±13
PI564869	390±30	2.0±0.7	2.1±0.7	2.7±0.6	2.4±1.0	54±4.3	103±46	0.15±0.04	130±27	593±29	332±31
PI564879	391±33	1.9±0.7	1.8±0.6	2.6±0.6	2.5±1.3	52±3.1	108±52	0.14±0.03	117±14	617±24	366±22
PI564880	369±25	2.3±0.7	2.2±0.7	2.8±1.0	2.7±1.3	56±3.2	108±59	0.14±0.03	118±23	581±24	331±23

PI578519	387±37	3.4±1.0	3.3±1.0	4.1±0.8	3.4±1.2	65±4.8	189±111	0.19±0.05	114±18	599±14	362±10
PI598641	353±16	2.9±0.9	3.0±0.9	3.7±0.4	2.9±1.1	65±4.7	160±75	0.16±0.04	113±23	575±20	352±21
PI628672	376±17	1.5±0.6	1.7±0.7	1.9±0.7	1.7±0.7	51±5.0	54±31	0.13±0.03	130±37	611±27	371±15
PI628683	375±35	1.9±0.7	1.8±0.6	2.4±0.7	2.2±1.0	49±5.8	90±54	0.17±0.04	126±24	589±23	354±14
PI634507	375±35	3.6±1.2	3.6±1.2	4.4±0.7	3.1±1.1	63±5.1	165±91	0.20±0.06	110±20	562±25	322±17
PI636511	436±43	1.7±0.9	2.0±0.9	2.0±1.2	2.1±1.2	49±17.1	66±47	0.18±0.05	126±20	591±32	340±22
PI639815	393±34	1.3±0.3	1.5±0.6	2.0±0.9	2.2±1.1	45±3.8	73±21	0.13±0.03	126±17	605±41	355±9
PI639849	375±40	3.0±0.8	2.6±1.0	3.8±1.0	3.3±1.1	61±17.1	124±86	0.19±0.07	116±13	583±41	350±34
PI662330	433±46	1.6±0.5	1.4±0.5	2.2±0.7	2.3±1.2	55±6.9	73±39	0.16±0.04	125±10	595±36	364±14
PI670374	371±34	1.6±0.5	1.9±0.7	1.9±0.7	2.3±0.7	56±6.4	108±56	0.13±0.04	107±12	613±19	368±14
W625134	350±14	3.1±0.7	3.0±0.8	3.6±0.6	3.0±0.7	65±3.2	148±69	0.15±0.05	123±19	594±13	366±20

Values represent mean of three-year data.

4.6.2 Association of crested wheatgrass accessions based on agro-morphological and nutritive traits

There were four principal components (PC1, PC2, PC3, and PC4) with eigenvalues great than 1, accounting for 52%, 18%, 11% and 10% of the total variation, respectively (Table 4.12). The first two components of the principle component analysis (PCA) explained approximately 70% of the total observed variation. The relative magnitude of eigenvectors in the first component (PC1) indicated that spring vigor, regrowth, hay stage vigor, plant height, and forage DM yield were the most important traits for classifying accessions into clusters (Table 4.12). The second component (PC2) was strongly correlated with NDF and ADF concentrations (Table 4.12). Leaf-to-stem ratio and crude protein concentration were the major variables that constituted the third component (PC3) (Table 4.12). Days to maturity was an important variable in the fourth component (PC4) (Table 4.12).

Table 4.12 Eigenvectors from the first four principal components for 11 traits used to classify 45 crested wheatgrass accessions

Variable	PC1[a]	PC2	PC3	PC4
Spring vigor	**0.41**	0.01	-0.09	0.06
Regrowth	**0.40**	0.09	-0.07	0.05
Hay stage vigor	**0.40**	0.02	0.09	0.01
Day to heading	0.01	-0.04	0.003	**-0.93**
Height	**0.37**	0.18	-0.11	0.01
DMY	**0.41**	0.04	0.02	0.01
Leaf-to-stem ratio	0.22	-0.18	**0.57**	-0.23
CP	-0.13	0.11	**0.79**	0.19
NDF	-0.07	**0.67**	0.09	-0.04
ADF	0.002	**0.68**	-0.01	-0.11
Leafiness	**0.38**	-0.07	0.14	0.10
Eigenvalue	5.72	1.93	1.18	1.09
Proportion (%)	51.95	17.57	10.76	9.89
Cumulative (%)	51.95	69.52	80.28	90.17

[a] The bold and underlined coefficient values indicate significant correlation with the relevant component in the PCA.

The relationships among the 45 crested wheatgrass accessions revealed by UPGMA cluster analyses based on 11 agro-morphological and nutritive traits are presented in Figure 4.7. Three main clusters were identified. The cluster I consists of 18 crested wheatgrass accessions with 12 of them being from Canada, and six other accessions, each originating from Germany, Ukraine, Russia, United States, Australia, and Kazakhstan. Plants in this cluster were tall and leafy, producing high DM yield (Table 4.13).

Table 4.13 Mean of agro-morphological and nutritive traits in 3 clusters grouped by cluster analysis of 45 crested wheatgrass accessions

Traits	Cluster I (n=18)	Cluster II (n=6)	Cluster III (n=21)
Day to heading (GDD)	494b[d]	441c	500a
Spring vigor	3.4a	2.9b	2.3c
Regrowth	2.5a	2.2a	2.6a
Hay stage vigor	3.2a	3.5a	2.9b
Leafiness	4.1a	3.4b	2.6c
Plant height (cm)	71.9a	62.2b	55.0c
Forage (DM) yield (g plant^{-1})	185a	136b	111b
Leaf-to-stem ratio	0.18b	0.18b	0.20a
CP[a] (g kg^{-1})	121a	123a	120a
NDF[b] (g kg^{-1})	597a	585a	588a
ADF[c] (g kg^{-1})	356a	350a	350a

[a] Crude protein concentration; [b] Neutral detergent fiber concentration; [c] Acid detergent fiber concentration

[d] Means with same letters within the row for each trait are not significantly different at $P = 0.05$.

The cluster II includes six crested wheatgrass accessions, two accessions from the United States, and four other accessions, each from Russia, China, Morocco, and Kazakhstan, respectively. Plants from this cluster were characterized by relatively early maturity (Table 4.12). The cluster III comprised of 21 accessions. Accessions in the third cluster had high leaf-to-stem ratio and late maturity (Table 4.12).

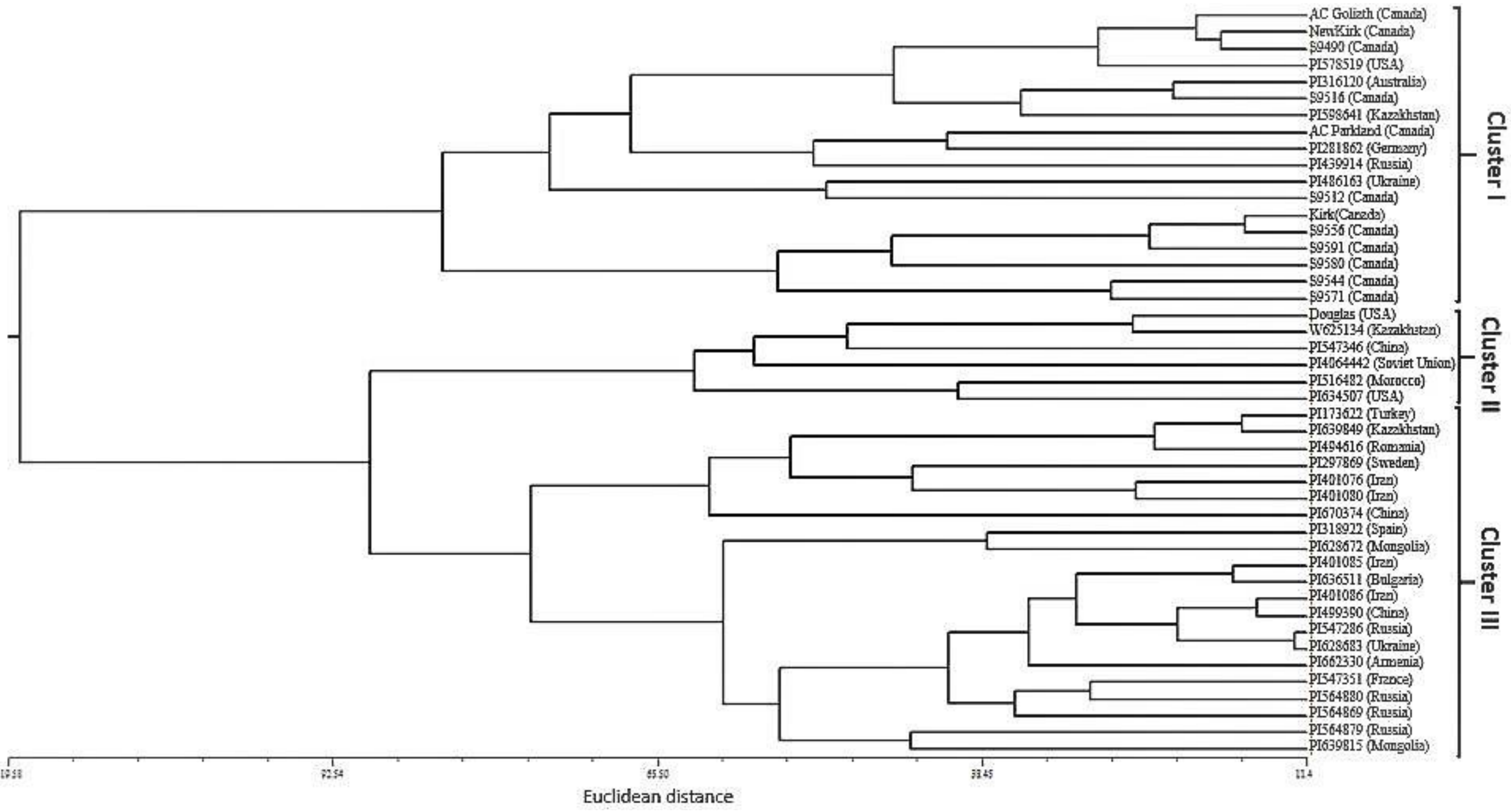


Figure 4.7 Dendogram of the 45 crested wheatgrass accessions revealed by UPGMA cluster analysis based on 11 agro-morphological and nutritive values.

4.7 Agro-morphological traits and nutritive value variation among crested wheatgrass accessions with different ploidy levels

4.7.1 Spring vigor, regrowth, hay stage vigor, and leafiness scores

Data for agronomic traits were further compared among different ploidy forms of crested wheatgrass. Spring vigor, regrowth, hay stage vigor, and leafiness scores were significantly *(*$P < 0.0001$*)* different among the three ploidy levels (Figure 4.8A-D). Diploid and tetraploid accessions had higher yielding, vigorous plants with more rapid regrowth than the plants in hexaploid populations, but no difference were found between diploid and tetraploid accessions. Leafiness score was the highest in diploid accessions, intermediate in tetraploid, and the lowest in hexaploid accessions.

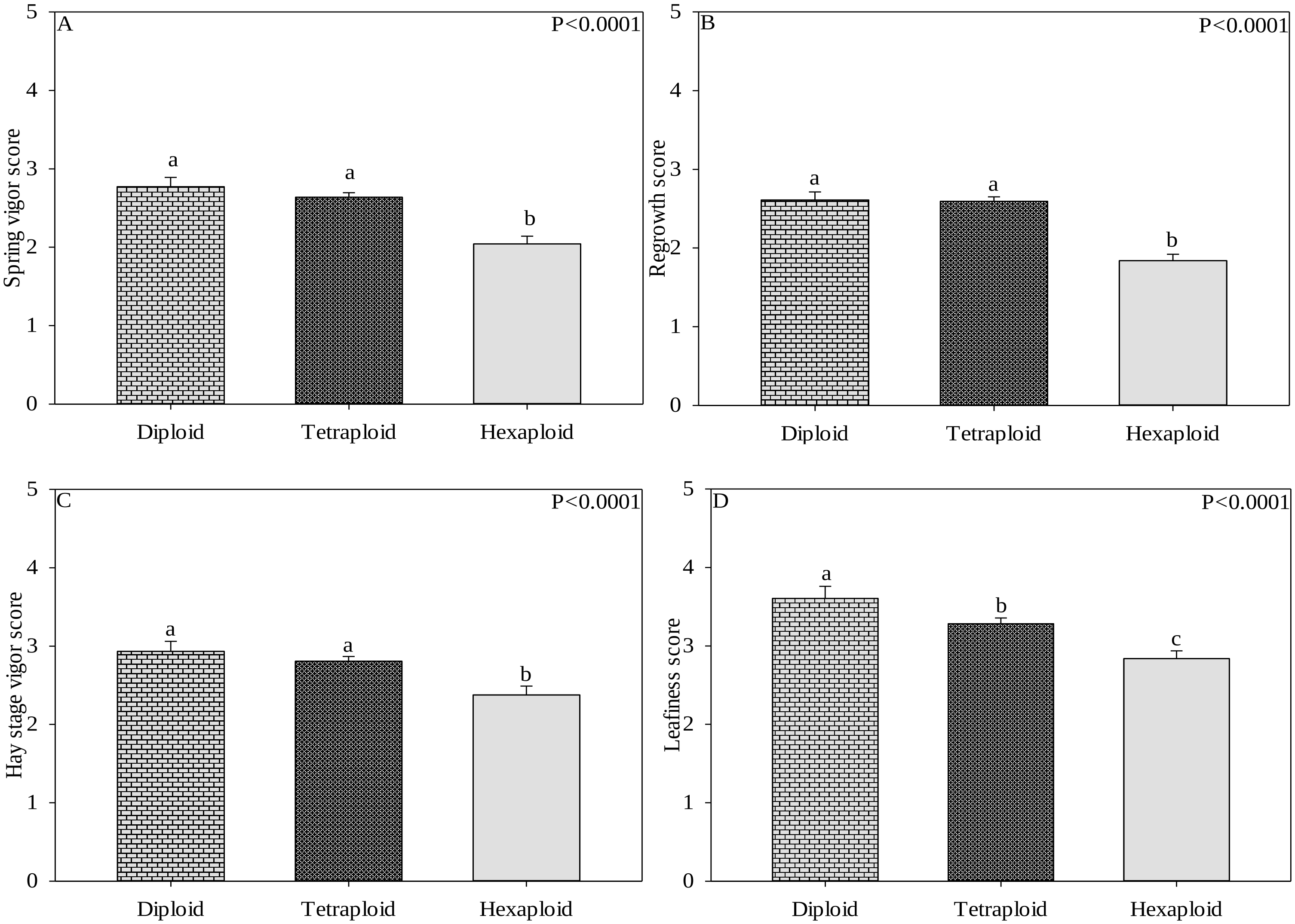


Figure 4.8 Three-year (2015, 2016 and 2017) mean of A) Spring vigor, B) Regrowth, C) Hay stage vigor, D) Leafiness scores among three ploidy forms of crested wheatgrass accessions at Saskatoon SK, Canada. (Bars represent means ± SE of 4 replicates). Means with different lower-case letters were significantly ($P \leq 0.05$) different among ploidy forms.

4.7.2 Days to heading, plant height, forage DM yield and leaf-to-stem ratio

There were significant differences among the three ploidy levels for days to heading ($P< 0.0001$), plant height ($P<0.0001$), DM yield ($P<0.0003$), leaf-to-stem ratio ($P=0.0008$) (Figure 4.9A-D). Compared to both diploid and tetraploid populations, hexaploid populations required significantly more days to reach maturity. There was no difference for days to maturity between diploid and tetraploid accessions. Tetraploid populations were significantly taller than diploid and hexaploid populations, but plant height was similar for the other two ploidy levels. Diploid and tetraploid populations had significantly higher forage DM yield as compared to hexaploid populations (Figure 4.9C). Both diploid and hexaploid populations had significantly higher leaf-to-stem ratio compared to tetraploid (Figure 4.9D), while it was similar between the diploid and hexaploid.

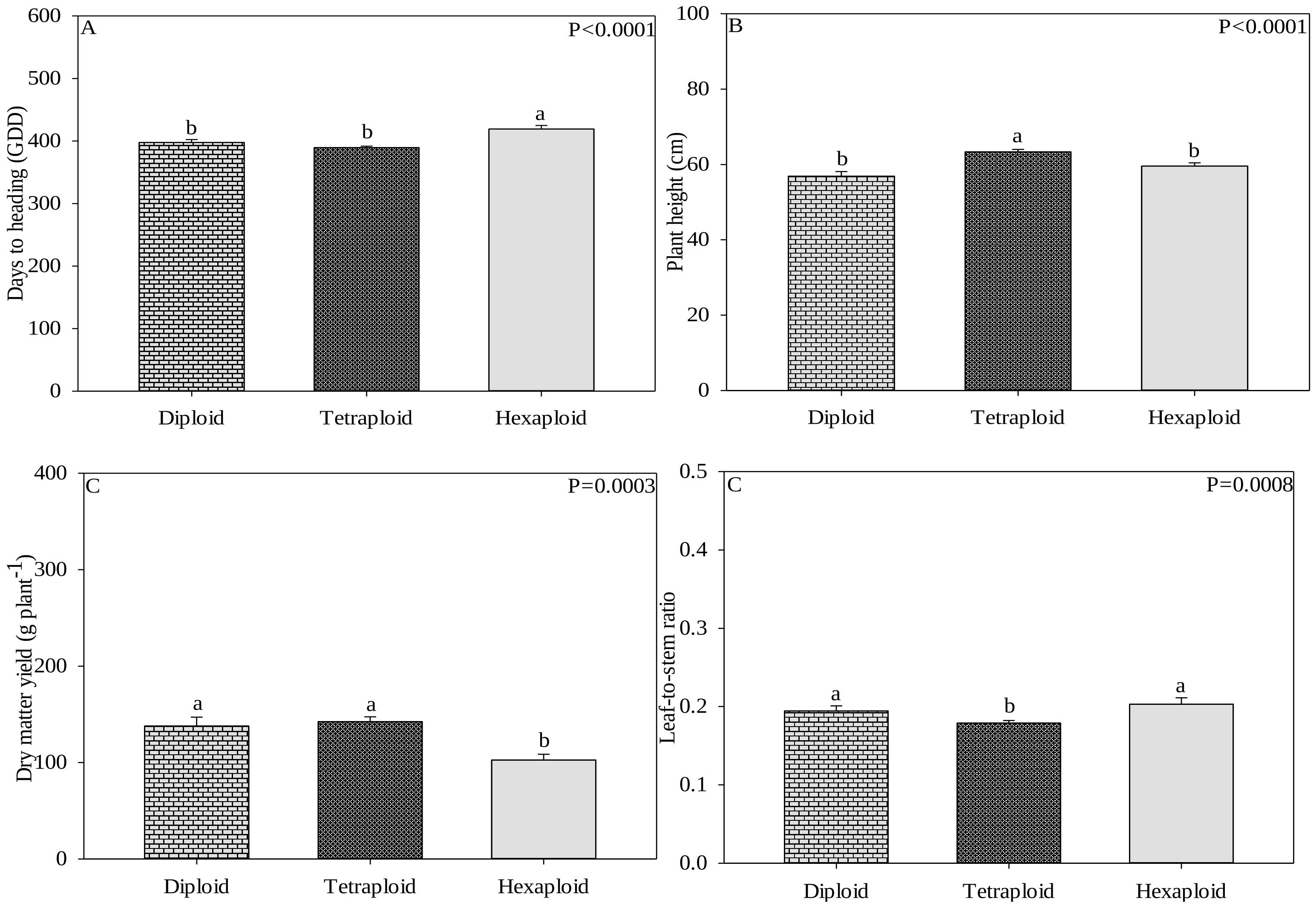
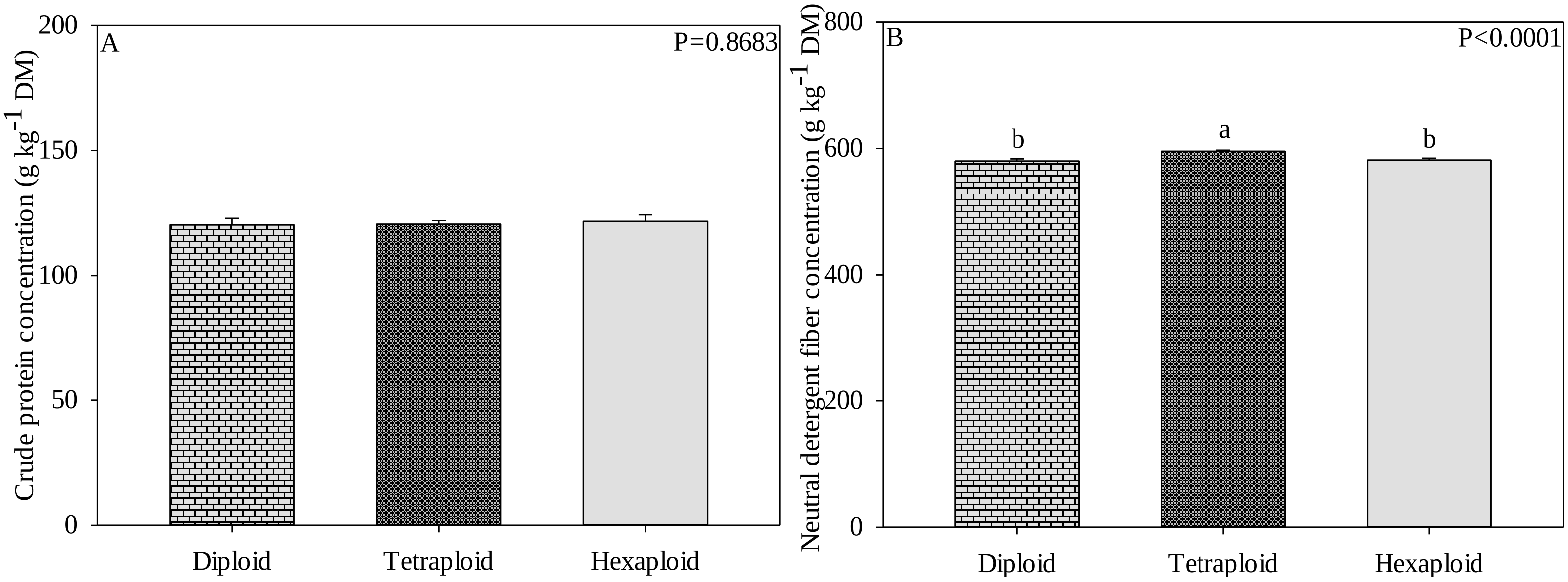


Figure 4.9 Three-year (2015,2016 and 2017) mean of A) Days to heading (GDD) , B) Plant height (cm), C) Forage DM yield (g plant^{-1}), and D) leaf-to-stem ratio among three ploidy forms of crested wheatgrass accessions at Saskatoon SK, Canada. (Bars represent means ± SE of 4 replicates). Means with different lower-case letters among ploidy levels were significantly different ($P \leq 0.05$).

4.7.3 Crude protein, neutral detergent fiber, and acid detergent fiber

Crude protein concentration was similar (*P*=0.8683) among the three ploidy levels of crested wheatgrass (Figure 4.10A). There were highly significant differences (*P*<0.0001) among ploidy levels for NDF with tetraploid accessions having higher NDF than diploid and hexaploid accessions (Figure 4.10B). Concentration of ADF was the lowest in diploid, intermediate in hexaploid, and the highest in tetraploid accessions (*P*<0.0001) (Figure 4.10C).



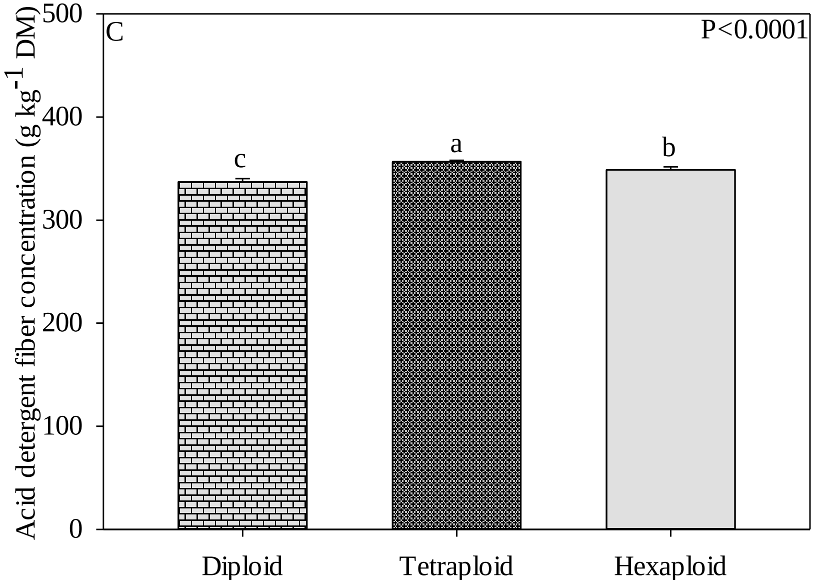


Figure 4.10 Two-year (2016 and 2017) mean of A) Crude protein (g kg^{-1}), B) Neutral detergent fiber (g kg^{-1}), and C) Acid detergent fiber (g kg^{-1}) concentrations among three ploidy levels of crested wheatgrass accessions at Saskatoon SK, Canada. (Bars represent means ± SE of 4 replicates).Means with different lower-case letters among ploidy levels were significantly different ($P \leq 0.05$).

4.8 Growth chamber experiment: Variation in days to heading and tiller number among selected germplasms of crested wheatgrass under a controlled growth environment

To further evaluate the days to heading, a study was conducted in the growth chamber using clones of selected crested wheatgrass accessions. In this study, days to heading showed a highly significant ($P < 0.0001$) difference among the five selected crested wheatgrass accessions, with the later maturing accession S9580 requiring the most days (769 GDD) compared to crested wheatgrass accessions AC Parkland, Kirk, PI516482 and PI439914 (Table 4.14). Days to heading ranged from 540 GDD (PI516482) to 769 GDD (S9580) for the selected accessions.

Tiller number varied significantly ($P=0.0007$) among the selected germplasms (Table 4.14). The mean tiller number at heading ranged from 44 tiller plant^{-1} (PI439914) to 96 tiller plant^{-1} (Kirk) with crested wheatgrass cultivars Kirk and AC Parkland having a significantly higher number of tillers than the accessions S9580, PI516482 and PI439914 (Table 4.14).

Table 4.14. Days to heading and tiller number among five selected crested wheatgrass accessions evaluated for height, tiller number and days to heading in Saskatoon SK, Canada

Accession	Days to heading (GDD)	Tiller number (No. plant^{-1})
AC Parkland	598	95
Kirk	545	96
S9580	769	59
PI516482	540	51
PI439914	551	44
LSD[a]	76.5	24.6
SEM[b]	130.0	16.4
P-value	<0.0001	0.0007

[a]Least significant difference at $P \leq 0.05$, [b]Standard error of means.

Plant height at heading was significantly ($P=0.0283$) different among the five selected crested wheatgrass accessions (Appendix D). High variation for plant height was detected in accession PI516482 (Appendix E). Variation range for tiller number was highest in accessions

Kirk and AC Parkland (Appendix E). The magnitude of variation for days to heading was the greatest in accession S9580 (Appendix E).

4.9 Discussion

In a breeding program, an assembly of diverse germplasm is the critical first step for the development of new cultivars (Poehlman and Sleper 1995). Usandizaga et al. (2015) indicated that local ecotypes, plant introductions, improved cultivars, and breeding lines can be genetic resources for increasing genetic diversity for further plant selection. In this thesis, I characterized a characterization of a collection of 45 crested wheatgrass accessions that varied according to their origin, ploidy level, and plant maturity. There was a particular interest to identify variation for plant maturity, and its impact on other forage characteristics.

Variation in maturity among perennial grasses has been considered an important indicator of forage quality and palatability (Humphreys 1989; Nekrosas 2003; Smith et al. 2005), thus, it is indirectly associated with livestock performance (Gowen et al. 2002). In crested wheatgrass, mature plants are coarse and unpalatable for the grazing animal (Hart et al. 1983), thus, development of later maturing crested wheatgrass cultivars would be useful to extend the grazing window in early summer. Baral et al. (2018) reported that a limited number of studies on genetic variability of germplasm is one of factors responsible for slow progress towards improvement of crested wheatgrass. Plant heading date is often used as an indicator for selection of plant maturity due to its high heritability and ease of assessment (Yano 200; Emebiri and Moody 2006). In the present study, days to heading varied greatly among the 45 accessions, differing more than 14 days between the earliest to latest genotypes. This indicates that further selection for plant maturity in crested wheatgrass is possible. In the present study, genetic diversity analysis on the 45 crested wheatgrass accessions was not conducted using molecular markers, however, an associated study by Baral et al. (2018) using genotyping-by-sequencing, showed high within and among genetic diversities in some of lines used in the study. In other studies, Mellish et al. (2002) using AFLP markers; Che et al. (2011); Che et al. (2015) using SSR marker reported a high degree of genetic diversity for crested wheatgrass accessions. In addition, certain breeding lines (i.e. S9580) selected for late maturity over a number of cycles had a late heading date both in the field and controlled environments in the study. Thus, phenotypic selection based on heading date is an effective selection method.

A number of conflicting results have been reported in forage grasses with respect to late maturity and forge DM yield (Ghesquiere et al. 2014; Sokolovic et al. 2016). In cocksfoot, Ghesquiere et al. (2014) reported that lateness was positively correlated with DM yield, however, Sokolovic et al. (2016) reported a decrease of DM yield with the delay of plant maturity. In the current study, plant DM yield and leafiness were positively associated with the delayed plant heading for the adapted Canadian accessions. For example, Canadian breeding lines S9516, S9571, and S9580, selected for late maturity, showed high forage DM yield and high leaf-to-stem ratios.

In this study, performance ranking of crested wheatgrass accessions were different among the three years, which can be explained in part by environmental variation. It was drier than normal during the growing seasons of 2015 and 2017, but an above normal rainfall was recorded in 2016. Mellish and Coulman (2002) reported that plant height and tiller density of crested wheatgrass can be reduced by low precipitation. Plants respond to drought stress by altering morphological and physiological characteristics such as maturing early, and reducing leaf area and plant height (Nilsen and Orcutt 1996; Shinozaki and Yamaguchi-Shinozaki 1997; Sanchez-Blanco et al. 2009). In other studies, drought stress also significantly reduced forage DM yield of grasses such as tall wheatgrass (Gazanchian et al. 2007), Kentucky bluegrass and perennial ryegrass (Pessarakli and Kopec 2008).

In general, North American accessions produced high DM yield and plants in these accessions were vigorous and tall compared to the accessions from Europe and Asia. This was not surprising because the North American accessions mainly consisted of Canadian cultivars and breeding lines, which were selected under western Canadian climatic and soil conditions. Smart et al. (2001) reported that, although leaf-to-stem ratio is an important quality indicator in grazing studies, selection of higher leaf-to-stem ratio data is difficult partly due to the tedious process involved in separating leaves and stems by hand. Results from this study revealed higher positive correlations of spring vigor, regrowth and hay stage vigor with dry matter yield, indicating the possibility of estimating dry matter yield by one or more of these visual ratings. Thus, visual rating can be used to improve dry matter yield instead of labor-intensive direct measurement of dry matter yield for thousands of plants. Similarly, spring growth score and dry matter yield showed high positive association with dry matter yield in nuttall’s alkali grass [*Puccinellia nuttalliana* (Shultes) Hitch.] (Liu and Coulman 2015).

Ploidy level has been known to affect agro-morphological characters of crested wheatgrass (Tai and Dewey 1966; Dewey and Asay 1975; Mellish and Coulman 2002). Reduction in tiller number by increasing ploidy level was reported in crested wheatgrass (Mellish and Coulman 2002), Russian wildrye [*Psathyrostachys juncea* (Fish. Nevski)] (Berdahl and Reis 1996) and orchardgrass (Bretagnolle and Lumbaert 1995), which was consistent with the findings from the present study. In our growth chamber study, with the exception of the tetraploid accession Kirk, all tetraploid accessions (S9580, PI516482 and PI439914) had lower tiller number than diploid accession AC Parkland. As expected, the tetraploid crested wheatgrass plants were taller than diploid plants in the current study, which was in consistent with the findings of Mellish and Coulman (2002). However, hexaploid accessions were shorter than tetraploids in our study, which in part may be related to poor adaption of hexaploid types for the environment of this study. No hexaploid cultivars are grown in western Canada, so all of the hexaploid accession included in this study were from other countries.

Alternation of plant characteristics due to ploidy differentiation also affected steer grazing behavior in crested wheatgrass (Iwaasa et al. 2014). Tetraploid crested wheatgrasses tend to have a more upright, coarse growth habit when compared with the finer, more decumbent tillers and leafy habit of diploid cultivars. However, a number of studies have reported that tetraploid crested wheatgrasses have been grazed preferentially compared to diploid crested wheatgrasses (Rogler 1944; Vogel et al 1993; Bruynooghe 1997; Iwaasa et al. 2014). Knowles and Kilcher (1983) explained that tetraploid wheatgrasses are more often grazed due to their reported upright and taller plant heights compared with the diploid crested wheatgrasses. Nevertheless, all crested wheatgrass accessions contained crude protein concentrations above 91 g kg^{-1} DM in 2016 and 2017, exceeding the 70 to 80 g kg^{-1} DM requirement of pregnant cows (Turner and Raleigh 1985; Van Soest 1994).

On the basis of agro-morphological and nutritive value, the 45 crested wheatgrass accessions were grouped into three main clusters. Accessions from Europe and Central Asia were the ones that exhibited the widest range of agronomic diversities as they were distributed in all three clusters. All North American accessions, with the exception of cultivar “Douglas” and accession PI634507 (“NU-ARD AC2”), were in cluster I. This is not surprising as the majority of Canadian lines were genetically associated with each other such as the cultivar “Newkirk” which was selected from progenies of crosses between cultivars “Kirk” and “AC Goliath” (Baral et al.

2018). Accession PI578519 (“Ruff”) from Nebraska, United States was in the first cluster, which may be because of its association with Canadian cultivar “Fairway”. Cultivar “Douglas” and PI634507 (“NU-ARD AC2”), both from the United States, were developed using accessions originating from Russia, Turkey, and Iran (Ogle 2006). Accessions W625134 and PI406442 originated from Kazakhstan and former USSR, which justify their close grouping in the second cluster (Asay 1992). The accessions in the third cluster had low spring vigor, were short and later maturing, which may also indicate their poor adaptation to the region of the present study.

5. 0 General discussion, summary and conclusions

Development of later maturing crested wheatgrass cultivars that would maintain their nutritive values into the summer grazing season is an important breeding goal in semi-arid regions of Canada. To achieve this goal, there are two possible breeding pathways: 1) population improvement by phenotypic recurrent selection with or without progeny tests if the source populations consisted of late maturing and adapted breeding lines (Hurst and Hall 2007). 2) introgression of genes governing late maturity to the existing breeding populations by crossing them with late maturing genotypes. This M.Sc. research project was a pre-breeding effort to characterize 45 crested wheatgrass accessions representing 18 different countries for plant maturity and their associated agronomic characteristics and nutritive value such as spring vigor, leafiness, hay stage vigor, regrowth, DM yield, plant height, leaf-to-stem ratio and nutritive value (CP, NDF and ADF) in a field nursery. In addition, certain selected early and late crested wheatgrass accessions were further evaluated for days to heading, plant height and tiller number under a controlled environment. In this study, DNA content and ploidy determination were conducted for all 45 crested wheatgrass accessions using flow cytometry.

Within a species, lines of different ploidy levels are known to be associated with plant morphology (Dewey and Asay 1975). Lines of different ploidy levels were consistent in field and growth chamber agronomic studies, with tetraploid plants being taller and larger compared to diploid plants. The hexaploid accessions in the field study had lower DM yield and plant height compared to diploid or tetraploid accessions; however, extremely wide leaves were observed in some hexaploid lines (Data not shown). As discussed previously, hexaploid accessions may be poorly adapted to the growing environment of our study.

Genome size of angiosperms was found to be in a range from 0.2 to 127.4 pg (Vogel et al. 1999; Palomino et al. 1999). The results of the present study showed that crested wheatgrass contained an intermediate size genome (ranging from 14.12 to 39.48 pg), which was in agreement with results of other studies (Vogel et Al. 1999; Yousofi and Aryavand 2004; Copete et al. 2018). Genome organization, plant evolution and ecological adaptation of germplasm are among the many areas of research where the exact knowledge of genome size is important; thus, this information obtained from the current research will be of importance in crested wheatgrass genetic and breeding studies. High genetic, ploidy and morphological diversities observed in the crested wheatgrass complex may be attributed to its outcrossing nature, high rate of inter-ploidy crossing and wide range of geographical distribution (Larson et al., 2001; Mellish et al. 2002; Che et al.2008; Che et al. 2011; Che et al. 2015; Baral et al. 2018; Copete et al. 2018).

Plant height and forage DM yield have been reported to be two of the most important factors that affect animal preferences in crested wheatgrass (Jones et al. 1994); therefore, it is always important to select tall and leafy plants with high biomass production during early growth stages that can potentially increase steer grazing preference of crested wheatgrass. In this study, the average plant height of crested wheatgrass accessions was 80 cm, however, individual plants in some Canadian accessions ranged from 100 cm (AC Goliath and S9571) to 106 cm (S9580). In general, North American accessions showed superior agronomic performance than the accessions from Europe or Asia, indicating a high regional adaptation for agro-morphological traits.

Three ploidy levels (diploid, tetraploid and hexaploid) were detected with majority of the accessions being tetraploid. Plant heading date and other measured agronomic characteristics varied among ploidy levels of crested wheatgrass in addition to the variation observed among and within accessions. Accessions with high genetic variation and superior agro-morphological traits could be useful in developing new synthetic varieties. Therefore, the first hypothesis “there will be variation in ploidy, which causes variation in plant maturity and other characteristics” and the second hypothesis “genetic variation exists within and among different crested wheatgrass cultivars and germplasm for plant maturity and other agronomic characteristics” were accepted. This research was conducted at one location in the dark brown soil zones , so it is unknown whether the performances of crested wheatgrass accessions will be the same in other zones in the Canadian Great Plains. A long-term field trial on crested wheatgrass at multiple locations would be useful for further characterization of genotype x environment interactions.

In summary, this study established a baseline information for many important traits of crested wheatgrass for future breeding and genetic improvement. The study also identified diploid, tetraploid and hexaploid levels of the 45 accessions with majority of the accessions being tetraploid. There were significant variations in plant maturity and other measured characteristics among the ploidy levels and among accessions. Significant genetic variation for late maturity exists among and within accessions in crested wheatgrass to produce accessions with high leaf-to-stem ratio, high DM yield, and tall plants. The breeding lines S9516, S9571 and S9580 were the most promising lines for late maturity.

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Appendix A.1 Flowering date range of 45 crested wheatgrass accessions in 2015, 2016 & 2017 in Saskatoon, SK, Canada

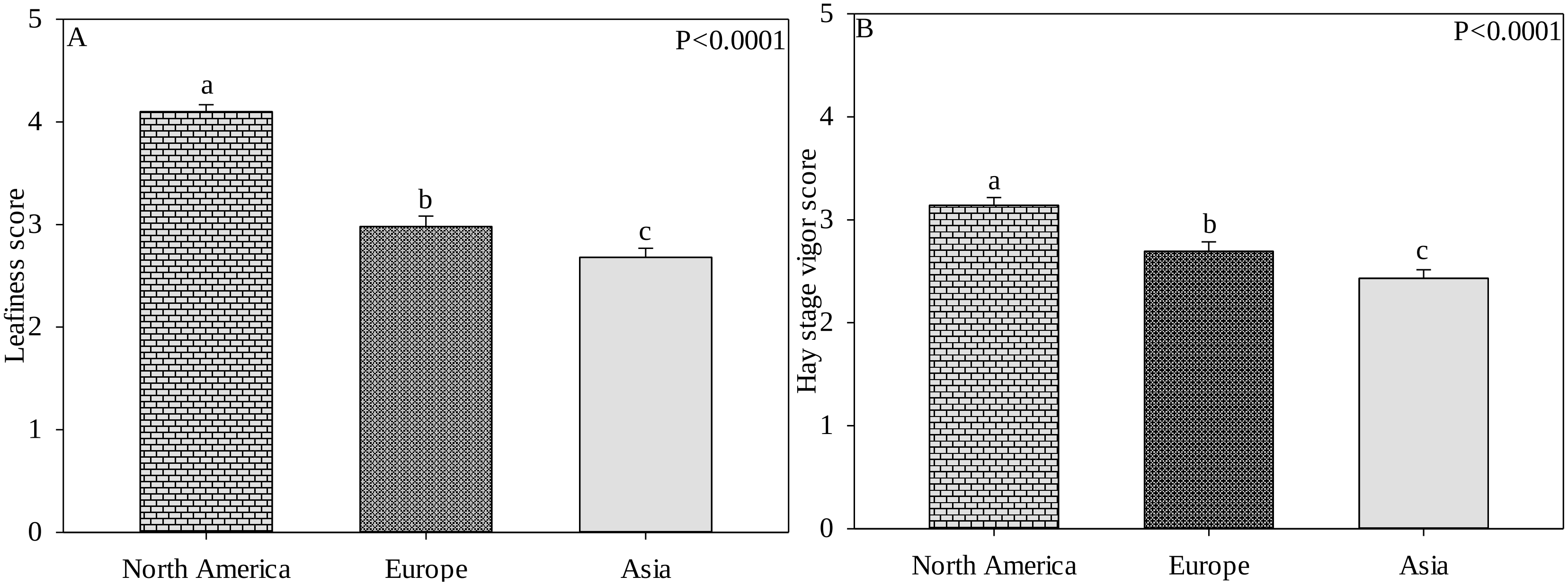
		Heading date range		
Accession	Country	2015	2016	2017
PI662330	Armenia	4-10 June	26-May-3-June	29-May-11-June
PI316120	Australia	3-4 June	26-31 May	29-May-5-June
PI636511	Bulgaria	6-15 June	26-May-8-June	29-May-12-June
AC Goliath	Canada	3-10 June	26-May-6-June	30-May-6-June
AC Parkland	Canada	3-8 June	26-May-3-June	29-May-7-June
Kirk	Canada	3-8 June	26-May-3-June	29-May-7-June
NewKirk	Canada	3-6 June	26-May-8-June	29-May-7-June
S9490	Canada	3-10 June	26-May-3-June	29-May-12-June
S9512	Canada	3-10 June	26-May-8-June	02-Jun-12-June
S9516	Canada	4-8 June	26-May-3-June	30-May-7-June
S9544	Canada	3-10 June	26-May-8-June	29-May-12-June
S9556	Canada	3-10 June	26-May-6-June	29-May-7-June
S9571	Canada	3-10 June	26-May-6-June	30-May-12-June
S9580	Canada	4-10 June	26-May-13-June	31-May-11-June
S9591	Canada	3-10 June	26-May-6-June	30-May-12-June
PI499390	China	3-8 June	26-May-3-June	29-May-12-June
PI547346	China	3-6 June	26-31-May	29-May-5-June
PI670374	China	3-8 June	26-31 May	29-May-7-June
PI547351	France	2-10 June	26-May-6-June	31-May-12-June
PI281862	Germany	4-8 June	26-May-8-June	29-May-12-June
PI401076	Iran	3-10 June	26-May-6-June	31-May-12-June
PI401080	Iran	4-10 June	26-May-6-June	29-May-11-June
PI401085	Iran	6-17 June	26-May-6-June	31-May-15-June
PI401086	Iran	3-8 June	26-May-3-June	31-May-7-June
PI598641	Kazakhstan	3-8 June	26-31 May	29-May-6-June
PI639849	Kazakhstan	4-8 June	26-31May	29-May-5-June
W625134	Kazakhstan	3-10 June	26-31 May	29-30 May
PI628672	Mongolia	4-8 June	26-May-01-June	29-May-6-June
PI639815	Mongolia	3-8 June	26-May-3-June	29-May-5-June
PI516482	Morocco	3-6 June	26-May	29-May-5-June
PI494616	Romania	3-8 June	26-31 May	29-May-6-June
PI439914	Russia	4-6 June	26-May	29-May
PI547286	Russia	3-10 June	26-May-6-June	29-May-11-June
PI564869	Russia	3-10 June	26-May-6-June	29-May-12-June
PI564879	Russia	3-10 June	26-May-6-June	29-May-6-June
PI564880	Russia	3-10 June	26-May-8-June	29-May-5-June
PI406442	Soviet Union	4-8 June	26-May-1-June	29-May-5-June
PI318922	Spain	3-10 June	26-May-8-June	29-May-7-June
PI297869	Sweden	3-8 June	26-May-6-June	29-May-6-June
PI173622	Turkey	4-8 June	26-May-3-June	29-May-12-June
PI486163	Ukraine	3-15 June	26-May-6-June	29-May-6-June
PI628683	Ukraine	4-8 June	26-31 May	29-May-6-June
Douglas	USA	3-8 June	26-May-3-June	29-May-7-June
PI578519	USA	3-8 June	26-31 May	29-May-7-June
PI634507	USA	3-10 June	26-31 May	30-May-6-June

Appendix A.2 Leafiness and hay stage vigor of 45 crested wheatgrass accessions in 2015, 2016 and 2017 at Saskatoon SK, Canada

			Leafiness[a]	Hay stage vigor[b]		
Accession	Country	Continent	2-year mean	2015	2016	2017
AC Goliath	Canada	North America	4.1	2.6	3.2	4.2
AC Parkland	Canada	North America	4.7	2.4	3.1	4.7
Kirk	Canada	North America	4.1	2.5	2.7	4.2
Newkirk	Canada	North America	3.8	2.9	2.5	3.8
S9490	Canada	North America	3.9	2.9	2.4	4.1
S9512	Canada	North America	3.8	2.5	3.0	4.2
S9516	Canada	North America	3.8	2.0	1.9	4.0
S9544	Canada	North America	4.6	2.8	2.2	4.5
S9556	Canada	North America	4.5	2.5	2.7	4.4
S9571	Canada	North America	4.3	2.7	2.8	4.7
S9580	Canada	North America	4.2	2.5	2.8	4.0
S9591	Canada	North America	4.1	2.5	2.7	4.2
Douglas	USA	North America	3.3	1.8	2.5	3.4
PI578519	USA	North America	4.1	2.4	3.4	4.6
PI634507	USA	North America	4.4	2.0	3.2	4.2
PI636511	Bulgaria	Europe	2.0	0.9	3.2	2.1
PI547351	France	Europe	3.5	1.7	3.3	3.9
PI281862	Germany	Europe	4.3	1.8	3.1	4.4
PI494616	Romania	Europe	3.7	1.2	3.5	4.1
PI439914	Russia Federation	Europe	3.9	2.2	4.3	4.3
PI547286	Russia Federation	Europe	2.9	1.2	3.4	2.8
PI564869	Russia Federation	Europe	2.7	1.4	3.0	3.0
PI564879	Russia Federation	Europe	2.6	1.3	3.3	3.0
PI564880	Russia Federation	Europe	2.8	1.3	3.3	3.4
PI406442	Soviet Union	Europe	2.6	1.2	3.8	3.1
PI318922	Spain	Europe	2.0	1.2	2.9	3.4
PI297869	Sweden	Europe	2.0	1.2	2.6	2.7
PI173622	Turkey	Europe	2.8	1.4	3.0	2.9
PI486163	Ukraine	Europe	4.7	2.1	4.2	4.6
PI628683	Ukraine	Europe	2.4	1.1	2.3	3.3
PI316120	Australia	Australia	3.7	2.2	2.7	3.5
PI662330	Armenia	Asia	1.9	1.2	2.9	2.8
PI499390	China	Asia	3.0	1.8	3.8	3.4
PI547346	China	Asia	2.2	1.1	3.6	2.1
PI670374	China	Asia	1.9	1.5	2.8	2.5
PI401076	Iran	Asia	2.7	1.5	3.0	2.4
PI401080	Iran	Asia	2.9	1.1	2.6	3.1
PI401085	Iran	Asia	2.8	1.0	2.8	2.3
PI401086	Iran	Asia	2.4	1.0	3.5	2.2
PI598641	Kazakhstan	Asia	3.7	1.9	3.0	4.0
PI639849	Kazakhstan	Asia	3.8	2.0	3.7	4.1
W625134	Kazakhstan	Asia	3.6	2.1	2.7	3.5
PI628672	Mongolia	Asia	1.8	1.1	2.0	2.1
PI639815	Mongolia	Asia	2.1	1.2	3.5	2.0
PI516482	Morocco	Africa	3.3	2.0	3.6	3.5
LSD[c] (0.05)			0.7	0.6	1.2	0.8
SEM[d]			0.3	0.2	0.4	0.3
CV[e]			26.6	34.3	17.1	23.2
P-value			<0.0001	<0.0001	0.037	<0.0001

[a] Leafiness rating scale 1-5 (1 = low percent of leaves, 5 = leafy plants); [b] Hay stage vigor rating scale 1-5 (plants with a low potential biomass yield, 5 = plants with a high potential biomass yield) [c] Least significant difference at $P<0.05$; [d] Standard error of means; [e] Coefficient of variation

Appendix A.3 Two-year means (2016 and 2017) of A) leafiness score and three-year means (2015, 2016 and 2017) of B) hay stage vigor score for crested wheatgrass accessions grown at Saskatoon SK, Canada (Bars represent means ± SE of 4 replicates). Means with different lower-case letters were significantly different (*P*≤0.05).



Appendix A.4 Plant height among 5 selected crested wheatgrass accessions evaluated in Saskatoon SK, Canada

Accession	Plant height (cm)
AC Parkland	29
Kirk	35
S9580	35
PI516482	34
PI439914	34
LSD[a]	3.8
SEM[b]	4.6
P-value	0.0283

[a] Least significant difference at $P \leq 0.05$, [b] Standard error of means.

Appendix A.5 Mean and standard deviation for plant height, tiller number and days to heading of 5 selected crested wheatgrass accessions evaluated in Saskatoon SK, Canada

Accession	Plant height (cm)	Tiller number (n)	Days to heading (GDD)
AC Parkland	29±4.69	95±52	598±180
Kirk	35±5.59	95±54	545±134
S9580	35±5.82	59±34	767±218
PI516482	33±9.97	52±44	540±141
PI439914	34±4.99	45±27	551±133