STUDY ON NITROGEN METABOLISM UNDER THE INFLUENCE OF DIFFERENT PLANTING DATES IN OAT (*Avena sativa* L.)

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
in
BIOCHEMISTRY
(Minor Subject: Botany)

By

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CERTIFICATE I

This is to certify that the thesis entitled, **"Study on nitrogen metabolism under the influence of different planting dates in oat (*Avena sativa* L.)"** submitted for the degree of **Master of Science**, in the subject of **Biochemistry** (Minor subject: **Botany**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Ms. Aanchaldeep Kaur (L-2018-BS-230-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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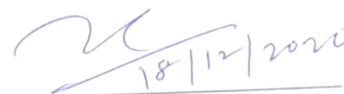

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ABSTRACT

The current investigation was carried out to evaluate the effect of staggered planting dates (Oct 9, Oct 24, Nov 13, Nov 26, Dec 11 and Dec 26) and growth stages (40, 50 and 60 DAS) in oat through field experiment during *Rabi* season using two genotypes. The OL-10 is a fast growing genotype with high regeneration potential and OL-11 is relatively slow growing single cut variety. The activities of nitrate reductase and nitrite reductase were observed high during optimum (Oct 24) and sub-optimum (Oct 9 and Nov 13) planting dates but low during late planting dates (Nov 26, Dec 11 and Dec 26). The activities of glutamine synthetase, glutamate synthase, glutamate dehydrogenase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase were observed high during late planting dates but were stable or slightly low at optimum and sub-optimum ones. The optimum and sub-optimum planting dates were also characterized by higher total chlorophyll, dry matter (DM), ash, crude fat, acid detergent fibre (ADF) and neutral detergent fibre (NDF) content in comparison with late planting dates. Crude protein, non-protein crude protein, *in vitro* dry matter digestibility, nitrate-N, nitrite-N and free amino acids increased with delay in planting dates. Meanwhile, the quality attributes of the oat except DM, ADF, NDF and free amino acids got declined with advancing maturity of vegetative fractions of plant. Furthermore, yield and yield attributes got declined with delay in planting time. Overall, OL-10 had higher nitrogen assimilation potential, nutritive value and yield potential as compared to OL-11 genotype.

Keywords: Digestibility, fodder yield, growth stages, nutritional composition, oat genotypes, planting dates.

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ਮੌਜੂਦਾ ਤਫ਼ਤੀਸ਼ ਖੇਤ ਦੇ ਤਜ਼ਰਬੇ ਦੇ ਜਰੀਏ ਜਵੀਂ ਵਿੱਚ ਫ਼ਸਲ ਲਗਾਉਣ ਦੀਆਂ ਵੱਖੋ-ਵੱਖ ਤਰੀਕਾਂ (9 ਅਕਤੂਬਰ, 24 ਅਕਤੂਬਰ, 13 ਨਵੰਬਰ, 26 ਨਵੰਬਰ, 11 ਦਸੰਬਰ ਅਤੇ 26 ਦਸੰਬਰ), ਵਿਕਾਸ ਦੇ ਪੜਾਅ (ਬੀਜਾਈ ਦੇ 40, 50 ਅਤੇ 60 ਦਿਨਾਂ ਬਾਅਦ) ਦੋ ਜੀਨੋਟਾਇਪ ਲੈ ਕੇ ਹਾੜ੍ਹੀ ਦੇ ਮੌਸਮ ਦੌਰਾਨ ਖੇਤ ਵਿੱਚ ਕੀਤੇ ਗਏ ਸਨ। OL-10 ਇੱਕ ਛੇਤੀ ਵੱਧਣ ਵਾਲਾ ਜੀਨੋਟਾਇਪ ਹੈ ਜੋ ਦੁਆਰਾ ਉਗਣ ਦੀ ਸਮਰੱਥਾ ਰੱਖਦਾ ਹੈ ਅਤੇ OL-11 ਤੁਲਨਾਤਮਕ ਤੌਰ ਤੇ ਹੌਲੀ ਵੱਧਣ ਵਾਲੀ ਸਿੰਗਲ ਕੱਟਾਈ ਵਾਲੀ ਕਿਸਮ ਹੈ। ਨਾਈਟ੍ਰੇਟ ਰਿਡਕਟੇਜ਼ ਅਤੇ ਨਾਈਟ੍ਰਾਈਟ ਰਿਡਕਟੇਜ਼ ਇੰਜ਼ਾਈਮਾਂ ਦੀਆਂ ਕਿਰਿਆਵਾਂ ਅਨੁਕੂਲ (24 ਅਕਤੂਬਰ) ਅਤੇ ਉਪ-ਅਨੁਕੂਲ (9 ਅਕਤੂਬਰ ਅਤੇ 13 ਨਵੰਬਰ) ਤਰੀਕਾਂ ਤੇ ਵੱਧ ਪਾਈਆਂ ਗਈਆਂ ਪਰ ਦੇਰ ਨਾਲ ਲਗਾਉਣ ਦੀਆਂ ਤਰੀਕਾਂ (26 ਨਵੰਬਰ, 11 ਦਸੰਬਰ ਅਤੇ 26 ਦਸੰਬਰ) ਵਿੱਚ ਘੱਟ ਪਾਈਆਂ ਗਈਆਂ। ਗਲੂਟਾਮਾਈਨ ਸਿੰਥੇਟੇਜ਼, ਗਲੂਟਾਮੇਟ ਸਿੰਥੇਜ਼ ਅਤੇ ਗਲੂਟਾਮੇਟ ਡੀਹਾਈਡ੍ਰੋਜੀਨੇਜ਼, ਗਲੂਟਾਮੇਟ ਓਗਜ਼ੇਲੋਐਸੀਟੇਟ ਟ੍ਰਾਂਸਐਮੀਨੇਜ਼ ਅਤੇ ਗਲੂਟਾਮੇਟ ਪਾਈਰੂਵੇਟ ਟ੍ਰਾਂਸਐਮੀਨੇਜ਼ ਇੰਜਾਈਮਾਂ ਦੀ ਕਿਰਿਆਵਾਂ ਫ਼ਸਲ ਨੂੰ ਦੇਰ ਨਾਲ ਲਾਉਣ ਦੀਆਂ ਤਰੀਕਾਂ ਵਿੱਚ ਵੱਧ ਸਨ ਪਰ ਅਨੁਕੂਲ ਅਤੇ ਉਪ-ਅਨੁਕੂਲ ਤਰੀਕਾਂ ਵਿੱਚ ਸਥਿਰ ਜਾਂ ਘੱਟ ਸਨ। ਕੁੱਲ ਕਲੋਰੋਫਿਲ, ਡ੍ਰਾਇਅ ਮੈਟਰ (DM), ਐਸ਼, ਕਰੂਡ ਫੈਟ, ਐਸਿਡ ਡੀਟਰਜੈਂਟ ਫਾਈਬਰ (ADF) ਅਤੇ ਨਿਊਟਰਲ ਡੀਟਰਜੈਂਟ ਫਾਈਬਰ (NDF) ਦੀ ਮਿਕਦਾਰ ਅਨੁਕੂਲ ਅਤੇ ਉਪ-ਅਨੁਕੂਲ ਤਰੀਕਾਂ ਵਿੱਚ ਵੱਧ ਪਾਈਆਂ ਗਈਆਂ। ਫ਼ਸਲ ਨੂੰ ਦੇਰ ਨਾਲ ਲਾਉਣ ਕਾਰਣ ਕਰੂਡ-ਪ੍ਰੋਟੀਨ, ਪ੍ਰੋਟੀਨ ਰਹਿਤ ਕਰੂਡ ਪ੍ਰੋਟੀਨ, ਇੰਨ ਵਿਟਰੋ ਸੁੱਕੇ ਮਾਦੇ ਦੀ ਪਾਚਣ ਸਮਰੱਥਾ, ਨਾਈਟ੍ਰੇਟ-ਐੱਨ, ਨਾਈਟ੍ਰਾਇਟ-ਐੱਨ ਅਤੇ ਫ੍ਰੀ ਐਮਾਈਨੋ ਐਸਿਡ (FAA) ਦੀ ਮਿਕਦਾਰ ਵਿੱਚ ਵਾਧਾ ਹੋਇਆ। ਇਸ ਦੌਰਾਨ DM, ADF, NDF ਅਤੇ FAA ਨੂੰ ਛੱਡ ਕੇ ਸਾਰੇ ਕੁਆਲਟੀ ਦੇ ਗੁਣ ਬੀਜਾਈ ਦੇ 60 ਦਿਨਾਂ ਤੇ ਘੱਟ ਪਾਏ ਗਏ। ਬੀਜਾਈ ਵਿੱਚ ਦੇਰੀ ਨਾਲ ਚਾਰੇ ਦੇ ਝਾੜ ਵਿੱਚ ਗਿਰਾਵਟ ਆਈ। ਕੁੱਲ ਮਿਲਾ ਕੇ OL-11 ਜੀਨੋਟਾਇਪ ਦੇ ਮੁਕਾਬਲੇ OL-10 ਵਿੱਚ ਨਾਈਟ੍ਰੋਜਨ ਸਮਰੂਪੀ ਸਮਰੱਥਾ, ਪੌਸ਼ਟਿਕ ਮੁੱਲ ਅਤੇ ਝਾੜ ਦੀ ਸਮਰੱਥਾ ਵੱਧ ਸੀ।

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ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ **ਵਿੱਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ**

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CHAPTER I

INTRODUCTION

Livestock plays an essential role in Indian economy. Livestock production has always been an integral part of the rural livelihood systems in India. About 8% of the country population is directly dependent on livestock husbandry for their livelihood (Tamizhkumran 2016). India's share is nearly 15% of the world's total livestock population (Kumari *et al* 2014). Though India is among the leading producers of milk and milk products, there is a huge pressure on the country to maintain the existing progress in milk production field (Kumar *et al* 2018). The fodders are an integral part of livestock and their production is the basic requirement of livestock industry. The productivity of our livestock often remains low due to inadequate supply of feed and fodder. In order to maintain the health of livestock, there is need for good quality green fodder (Rashid *et al* 2019).

Oat is a major cereal forage crop cultivated in the northern, central and eastern regions of India during *Rabi* season. Oat, sown in winter, have its own definite requirements for light and temperature for emergence, growth and flowering (Dabre *et al* 1993). It belongs to Poaceae family and ranked sixth in the world cereal production. It is a widely accepted crop due to its excellent growth patterns, rapid regrowth and better yield potential (Kumari *et al* 2014). It requires cool and moist climate for its growth and is utilized both for forage and seed production. The total coverage area of fodder crops in India is 8.3 million hectare, among which oat is one of the leading crop (ICAR 2017). The fodder oat was grown on 1.05 lac hectares in 2018-19 in Punjab (Package of Practices 2020-21).

Nitrogen is one of the most yield limiting plant nutrients under most agro ecological conditions (Fageria and Baligar 2005). It plays a vital role in photosynthesis and is an indispensable part of protein. The assimilation of nitrogen is directly responsible for crop biomass production and grain yield (Bergamo *et al* 2007). Nitrogen metabolism is one of the primary process of plant physiology that play a great role in maintaining growth and development of the plant (Ashraf *et al* 2018). The activities of N assimilating enzymes controls many cellular activities in plants and is necessary for stress tolerance (Singh *et al* 2016). Nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase (GS), glutamate dehydrogenase (GDH), glutamate synthase (GOGAT), glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) are all crucial enzymes involved in N metabolism and their activities helps in evaluating plant N status (Rachana *et al* 2018). NR is the most important rate-limiting enzyme and is the regulating point among the nitrogen metabolism enzyme. Nitrate reduction to nitrite is mediated by nitrate reductase and nitrite is further reduced to ammonia by nitrite reductase (Setif *et al* 2009). Ammonia is then converted into organic form glutamine by the rate limiting enzyme of ammonia assimilation glutamine synthetase and in turn levels of α-ketoglutarate are equilibrated by GOGAT and GDH activity

(Marcondes and Lernos 2012). The plastidic ferredoxin dependent enzyme GOGAT catalyses the reaction in which glutamine reacts with α-ketoglutarate to form glutamate (Mokhele *et al* 2012). GDH is the main enzyme for primary N assimilation which catalyses the deamination of glutamate to provide energy and to return carbon skeletons from amino acids to the reactions of carbon metabolism (Miflin 2002). The biosynthesis of N carrying amino acids and proteins is carried out by GOT and GPT and these also serve as markers of nitrogen use efficiency (Asthir *et al* 2018). GPT and GOT are also known as alanine aminotransferase and aspartate aminotransferase and these enzymes acts in the direction of deamination to provide amino acids, especially glutamic acid, for the common N-pool.

Oat and several other crop plants take up nitrogen mostly in the form of nitrate or ammonium and then convert to various amino acids (Giagnoni *et al* 2016). Nitrate is present in almost all plants, but it mainly becomes toxic in forages grown in the presence of extreme stress (Basso and Ritchie 2005). A sudden decline in plant growth under any stressful condition causes accumulation of nitrates in the plant. Apart from the nitrogen fertilizers, detrimental weather, herbicides, diseases, imbalance of soil nutrients, inadequate exposure to sunlight etc are some of the factors causing nitrate toxicity. The major source of nitrogen to the plant is through soil, but the nitrate content is more affected by the unfavourable weather conditions and different plant species (Mishra 2011). Developing leaves are the major site for the formation of plant products from nitrates. The leaf area of the plant may also be damaged or reduced by frost and hail. This may lead to reduction in the photosynthesis of the plant and also limits the absorption of nitrates by the roots, therefore nitrate may not be efficiently converted to plant proteins and in turn may get accumulated in the stem (Kaur and Goyal 2016). The level of unutilized nitrates on the plant surface mainly serve as a rich source of nitrate in animal diet (Kamra *et al* 2015). Normally, these plants are consumed by ruminant animals, the nitrate breaks down to ammonia and then gets converted into microbial protein by the bacteria present in the rumen (Kozloski 2009). But when ingested in higher amounts, accumulation of nitrite in the rumen may occur, which is an intermediate of nitrate to ammonia conversion. The absorption of nitrite by animal's blood system converts haemoglobin to methaemoglobin, a complex which is not capable of carrying oxygen from the lungs to tissues. Due to this, release of oxygen to various body tissues become difficult resulting in tissue asphyxiation and death of the animal (Benjamin 2006). Nitrate-N levels higher than 0.2% (2000 ppm) in animal feed are potentially disastrous to the health and productivity of ruminants (Kaur and Goyal 2016).

Staggered planting is one of the important influential factor that determines the response of the crop with seasonal variations. Weather conditions during variable planting dates influences nitrogen metabolic enzymes. The activity of nitrate reductase changes with respect to light and temperature (Shu *et al* 2016). The NR and NiR enzyme activity depends upon the reductants provided by the photosynthetic activity of plants (Bian *et al* 2020).

GS/GOGAT pathway is highly regulated by the temperature prevailing during the growing period (Lu *et al* 2005). GDH pathway remains stable or slightly increased under low temperature stress (Liu *et al* 2017). The enzyme activities remains low during early growth stages due to fog and low intensity of sunlight but as the growth continues the enzymes become more active due to presence of energy source in the form of sunlight (Kaur and Goyal 2016).

The impact of environmental variability is being seen on agricultural crops in the last few decades. Many recent studies have linked several climatic attributes such as temperature and sunshine hours with the nutritive value as well as yield of fodder crops (Dhillon *et al* 2019 and Salama 2019). Such researches were providing clues about the negative externalities of some weather conditions on yield of crops. Farmers often do staggered sowing to obtain products for longer period of time irrespective of the quality and quantity of the crop. However, plant scientists are more interested in estimating the quality traits of the crop to enhance the livestock productivity. The nutritional status of a forage crop is chiefly based upon the concentration of proteins, fats and carbohydrates. The composition of these organic nutrients determines the digestibility of forages which along with vitamins and minerals maintains the body functions of ruminant animals (Capstaff and Miller 2018). *In vitro* dry matter digestibility (IVDMD) evaluates the extent upto which feed component is digested by an animal through the microbial activity present in rumen (Barahona *et al* 2003). The pioneering works of Marten *et al* (1988) and Van Soest (1994) showed that fibre content was negatively correlated with the digestibility of crops. Most of the dry matter content present in plants consists of carbohydrates predominantly the cell wall polysaccharides such as cellulose and hemicellulose. Such carbohydrates make up the fibre content of fodder crops. In general, acid detergent fibre (ADF) content is known to determine the digestibility of the fodder crops and neutral detergent fibre (NDF) content estimates the intake potential of the ruminants (Bao *et al* 2007). It is well known that nitrogen is mainly provided to ruminants from the forage proteinsas they are vital for the formation of their own body proteins. Oat are considered to be the best source of proteins containing all the essential amino acids like threonine, lysine and methionine (Meydani 2009). Other nitrogen compounds found in forages such as amides, amines that are not the part of polypeptides are regarded as non-protein crude protein (NPCP). To illustrate the nutritive value further, ether extract consists of fats, waxes, oils, organic acids, pigments, sterols and vitamins A, D, E and K generally range from 3-4% in oat. In addition, ash content which is essential for the bone formation of livestock is rich in mineral content (Dhillon *et al* 2019). Any factor which affects the growth and development of plant surely influences its quality traits. Hence, the nutritive value of fodder crops depends upon the environment in which plant grows (Nahar *et al* 2015). Chlorophyll level is an important index used to evaluate the photosynthetic capacity of plants. Temperature variation during crop life cycle alters the activities of chlorophyll biosynthetic enzymes, thereby altering chlorophyll

content (Aghaee *et al* 2011).

Stage of harvest is also one of the likely nutritional quality determinants and some authors have considered it the most important factor affecting quality traits. The strong evidence of decline in crude protein, ash and ether extract content with advancing age of the crop was provided by many researchers (Malik *et al* 2015, Kaur and Goyal 2017 and Kaur *et al* 2013). Contrastingly, the cell wall constituents i.e. ADF and NDF content increases with plant maturity because N is replaced by fibre and lignin (Opsi *et al* 2012). Furthermore, the IVDMD content decreases with maturity of the fodder crops because of enhancement in lignin concentration (Lewis *et al* 2004). Nitrate content generally are higher in developing plants and the level decreases with plant maturity (Radiositis *et al* 2000).

In addition to the need of optimal nutritional content, rapid vegetative biomass accumulation is also the most desirable trait of a good fodder crop. Some authors correlates plant height with biomass of most crops and this factor together with number of tillers are underpinning attributes to assess yield of fodder crops (Mumtaz *et al* 2015 and Shahzad *et al* 2002). Low temperature is one of the most severe weather events that destructively affects dry matter yield and ultimately the crude protein yield (Shahzad *et al* 2002).

The genotypes are the monetary input which plays an essential role in the production of qualitative as well as quantitative fodder (Hameed *et al* 2014). There is definitely variability in the growth rate of genotypes at variable climatic conditions and consequently, in their adaptation to the cool climate. The genotypes having high biomass is always preferred for fodder production (Kumar *et al* 2017). The oat genotypes also varied in relation to nitrate and ammonia assimilating enzymes (Shah *et al* 2020). This is due to different rate of uptake and accumulation of nitrate ions and assimilation by various genotypes (Kaur *et al* 2015). Nitrogen efficient genotypes of wheat differed in all aspects of N metabolism (Chandna *et al* 2011). Therefore, selection of high yielding varieties with midrange of adaptability to edaphic and environmental conditions is very essential to increase yield per hectare (Tahir *et al* 2009).

Number of reports showed a direct correlation between N inputs and activity of nitrate reductase in plants (Kaur and Goyal 2016). Our knowledge on N uptake and its successive assimilation under different planting dates is still insufficient at biochemical and physiological level. Therefore, the present study is carried out on nitrogen assimilating enzymes in oat genotypes under the influence of different planting dates and harvesting stage. The main objectives of present study:

(i) To study nitrogen metabolism under the influence of different planting dates in oat.

(ii) To evaluate nutritional quality and yield parameters under different planting dates in oat.

CHAPTER II

REVIEW OF LITERATURE

Oat is a *Rabi* crop sown in winter season and considered to be one of the best dual purpose crops that fit well into the platter of human and cattle as well. The green fodder availability of oat can be manipulated by adjusting the time of planting as planting date was considered one of the essential yield contributing factor. Time of planting is mainly governed by weather conditions which affects many biochemical and physiological parameters like nitrogen metabolism enzymes, quality traits as well as yield of oat. Oat grows well in cool and moist climate as it requires more moisure to produce a given unit of dry matter than all other cereals but sometimes its growth is restricted in the months of December and Januuary because of low temperature and less available sunshine hours.

Nitrogen metabolic enzymes are affected by many factors like sunlight, frost, hail etc. Nitrate reductaseactivity is directly related to nitrate-N content in crops (Kaur and Goyal 2016). Nitrate poisoning generally encountered during the extreme environmental conditions when crops are affected by cold, frost, hail and low intensity of sunlight (Sidhu *et al* 2011). Ruminants like cattle, goat and sheep are more prone to nitrate toxicity. Affected animals show clinical signs when 20% of hemoglobin is converted to methemoglobin and death results when it reaches to 60-80% (Qudah *et al* 2009). Nitrate uptake and utilization in crops is of major value with respect to both environmental concern and quality of crop products.

Oat has a well-balanced nutritional composition as it chiefly contains many valuable nutrients such as carbohydrates, proteins as well as fats (Head *et al* 2010). Oat is unique in having high protein content of 11-15%. The required concentrations of quality traits such as crude protein, dry matter, crude fat, ash content, acid detergent fibre (ADF) and neutral detergent fibre (NDF) contents can be achieved by use of genetically improved cultivars as well as harvesting at optimum time (Cecelia *et al* 2007). Growth stage is an important influential factor determining oat quality and quality of oat decreases with advancing age of vegetative fractions of plant (Malik *et al* 2015). In general, fiber content of the fodder crops increases while proteins and digestibility decreaseswith advancing age of crops (Ball *et al* 2001). Fodders with lower ADF and NDF contents and higher protein content are easily digestible by ruminants (Kumar *et al* 2018).

Keeping all these points in mind, the review of literature has been divided into following main headings and subheadings.

2.1 Status of nitrogen metabolism enzymes in oat

2.1.1 Nitrate assimilation

2.1.2 Ammonia assimilation

2.1.3 Transaminases

2.2 Nutritional composition and digestibility in oat

2.3 Yield and yield components in oat

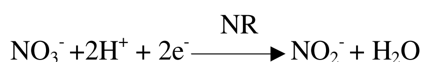
2.1 Status of nitrogen metabolism enzymes in oat

2.1.1 Nitrate assimilation

The assimilatory pathway of nitrate is a vital biological process in higher plants as it is the principal route by which inorganic nitrogen is incorporated into organic compounds. Nitrate assimilation is a highly regulated process because of its dependence on photosynthesis for energy and reductants of this pathway, nitrate and ammonium (Ali *et al* 2007).

2.1.1.1 Nitrate Reductase (NR, EC 1.6.6.1)

Nitrate Reductase normally catalyses the NADH-dependent two-electron reduction of nitrate (+5) to nitrite (+3). The soluble enzyme is located mainly in the cytosol, but existence of some plasma membrane-bound analogue has also been reported (Kaiser *et al* 2011). Nitrate reductase (NR), the rate-limiting enzyme usually found in both root andshoot tissues of plant. Although most NRs use NADH, some can use either NADH or NADPH (Beyzari *et al* 2014).



$$NO_3^- + 2H^+ + 2e^- \xrightarrow{NR} NO_2^- + H_2O$$

NR exists as homodimer of 100-110 kDa subunits (Marquez *et al* 2005). The native dimer consists of two identical subunits, each subunit consisting ofthree redox cofactors: FAD, heme b_{557}, and molybdenum. The electrons are transferred from the nicotinamide coenzyme to FAD, then to iron-heme, and finally to a molybdenum-pterin cofactor, which reduces the nitrate. Two structurally flexible ‘hinge’ regions on both sides of the haeme domain are connecting it with the Mo-MPT (hinge 1) and with the FAD domain (hinge 2). The dimer interface is located between hinge 1 and Mo-MPT (Campbell 2002). The monomeric subunit of cytosolic NR has two active sites, one for NADH to donate electrons and another for reduction of nitrate to nitrite. In addition to its basic role in catalysing a two-electron transfer from NADH to nitrate, NR has been shown to also catalyse one-electron transfer reactions. Nitrite can be reduced to NO and as the reaction is competitively inhibited by nitrate (Rockel *et al* 2002), it probably also occurs at the nitrate reduction site of the Mo-MPT centre. Phosphorylation as such does not change NR activity, but binding of a 14-3-3 dimer to the phosphorylation motif in the presence of Mg^{2+} renders NR inactive. In leaves, light or photosynthesis appears as a major trigger for activating NR by Ser dephosphorylation (Campbell 2002).

Light induction of NR is mediated not only by photosynthesis but also by phytochrome (Kaiser *et al* 2011). In the *Arabidopsis* phytochrome-deficient double mutant, the diurnal modulation of NR levels were abolished and NR levels remained very low (Jonassen *et al* 2008). The light response of NR shows that the enzyme is only synthesized if substrate (NO_3^-) and carbon skeletons for amino acid synthesis are available. When plants are

grown on ammonium, instead of nitrate, their extractable NR activity in leaves is usually below the detection limit (Planchet *et al* 2005). NR activity declined in the leaves pretreated with excess nitrate due to feedback inhibition of higher concentration of nitrate-N (Zhang *et al* 2017). Shading inhibited NR activity in leaf stalks and blades of green leaves (Wojciechowska and Siwek 2006). In an earlier study, NR activity in rice was uplifted almost 20-fold in the presence of light and nitrate but there was a gradual decrease in the activity in the absence of light (Ali *et al* 2007). A recent study reported the influence of seasonal variation on nitrate reductase activity (Prakash and Saxena 2020).

In an earlier study on oats, NR activity was lowest during early growth stages due to cool weather, fog and low intensity of light and the highest activity was observed at 60 DAS (Kaur and Goyal 2016). Previous reports using maize stated a decreasing trend of nitrate uptake and assimilation with ageing of plant (Hirel *et al* 2005). According to Hirel *et al* (2001), NR activities remained low during the vegetative state in maize so that NO_3^- formed could stay in the vacuoles which can be later remobilized for the production of grain and protein.

NR activity differs among varieties of cereal crops (Chandna *et al* 2010). According to Shah *et al* (2017), it was reported that NR activity was maximum in ZD9 genotype of barley and lowest in XZ56. In oat, the highest NR activity was reported in OL-9 genotype and lowest in OL-125 (Kaur and Goyal 2016). The activity of NR in leaves is dependent on the source of N and continuous supply of nitrate through xylem (Cazetta and Villela 2004).

2.1.1.2 Nitrite Reductase (NiR, EC 1.7.7.1)

NiR is a plastidic enzyme which catalyzes a six-electron reduction of nitrite to ammonium supplied by reduced ferredoxin (Meyer and Stitt 2001). NiR is found in the chloroplasts of green tissues and in the plastids of non-green tissues of plants.

$$NO_2^- + 8H^+ + 6e^- \xrightarrow{\text{NiR}} NH_4^+ + 2H_2O$$

The enzyme exists as a monomer having a molecular mass of 65.4 kDa which includes a ferredoxin like hemoprotein, 4 Fe-S clusters and a siroheme binding site (Wang *et al* 2015). The electrons from ferredoxin are first transferred to the 4 Fe-S cluster of nitrite reductase and then to siroheme, finally to the substrate which remains bound until its complete reduction to ammonia (Kuznetsova *et al* 2004). Due to the toxicity of nitrite, the activity of these two enzymes, nitrate reductase and nitrite reductase must be in coordination so that there is no build-up of the intermediate. Various environmental factors regulated the gene expression of these nitrate assimilating enzymes and their activities. Examination of NiR regulation showed that NiR activity was regulated by nitrate, light and plastids (Mohr *et al* 1992). In leaves, reduction of nitrite to ammonia via the chloroplast electron transport chain and ferredoxin (Fd)-nitrite reductase is very low in the dark (Kaiser *et al* 2011). Normally,

light dependent nitrite reduction declined nitrite concentrations, not only within the plastids, but also in the cytosol. The rate of nitrite conversion to ammonium depends on the suppressive effects exerted by reduced nitrogen metabolites such as ammonium and amino acids (Aslam *et al* 1996). In an earlier study on rice, NiR activity was uplifted 3-fold in the presence of light and nitrate (Ali *et al* 2007).

A pioneer study reported that mature leaves of corn had high NiR activity as compared to younger ones and opposite in case of NR (Kenis *et al* 1992). Kaur *et al* (2015) suggested that NR activity varied from anthesis, tillering and post-anthesis stage in wheat. Kaur and Goyal (2016) recorded that NiR activity was highest at 60 DAS and minimum at 30 DAS in oat. A recent study on wheat showed that NiR activity was found to be different at variable growth stages and Shinong 086 showed higher activity in comparison with Jimai 325 (Lin *et al* 2020).

In a previous study on barley, NiR activity differed among nitrogen efficient genotypes and nitrogen inefficient genotypes (Shah *et al* 2017). A recent study claimed that NiR activity was found to be comparable in two genotypes of wheat (Fortunato *et al* 2019). However, NiR activity showed significant differences among genotypes of Brassica under nitrogen stressed and nitrogen sufficient conditions (Gupta *et al* 2019). According to Chandna *et al* (2011), it was found that low N-efficient wheat genotypes are not utilizing but rather accumulating the excess nitrate that is being supplied to them.

2.1.2 Ammonia assimilation

Ammonium formed from nitrate is toxic if it accumulates in large amounts so its further assimilation is very essential. Ammonia is incorporated into its organic molecules, glutamate and glutamine by the action of three enzymes:

- Glutamine synthetase (GS, EC 6.3.1.2)
- Glutamate synthase (GOGAT, EC 1.4.1.14)
- Glutamate dehydrogenase (GDH, EC 1.4.1.2)

2.1.2.1 Glutamine synthetase (GS, EC 6.3.1.2)

Glutamine synthetase is the central enzyme which catalyses the conversion of carboxyl group of glutamate to glutamine through ATP dependent fixation of NH_4^+ (Guan *et al* 2014). It requires a divalent cation such as Mn^{2+}, Mg^{2+} or Co^{2+} as a cofactor (Coleman *et al* 2012).

$$\text{L-Glutamine} + NH_4^+ + \text{ATP} \xrightarrow{\text{GS}} \text{L-Glutamine} + \text{ADP} + \text{Pi}$$

GS is an octameric enzyme having molecular weight of 350-400 kDa and it may be either heteromeric or homomeric. It consists of two isoenzymes:

- Cytosolic GS (GS1)
- Plastidic GS (GS2)

GS1, having molecular weight of 38-40 kDa is being encoded by small subfamily of

genes which varied from three in rice (Tabuchi *et al* 2005), five in maize (Martin *et al* 2006) and seven in wheat (Bernard *et al* 2008). GS2, having molecular weight of 42-45 kDa is encoded by only one gene (Hirel *et al* 2007). GS1 is localised in the cytoplasm of non-green tissues such as flowers, seeds, roots and nodules and also in the phloem companion cells of leaves (Unno *et al* 2006). GS2 is located in the chloroplasts of photosynthetic tissues and plastids of roots and etiolated roots (Sunrez *et al* 2002). GS1 is induced in leaves during senescence and function primarily in the ammonia reassimilation released from catabolism of amino acids during biotic and abiotic stress (Bernard and Habash 2009). GS1 is also found to be present in the mesophyll cells of barley (Tobin and Yamaya 2001). The role of GS2 is to assimilate ammonia released from nitrate reduction as well as formed during the photorespiration process (Masclaux-Daubresse *et al* 2010).

The relative proportions of GS1 and GS2 varied depending upon plant species organ, the developmental stage of the particular organ and under the wide range of environments. This might ensures a rapid reassimilation of ammonium derived from multiple sources (Miflin and Habash 2002). The important factors affecting the GS enzyme activity are light, carbon status and nitrogen nutrition (Oliveira *et al* 2001). Miflin and Habash (2002) suggested that there was no significant effect of light on the activity of GS1. In another study, it was reported that GS2 gene transcription and protein synthesis has been found to be regulated by light in photosynthetic organs involving the action of UV-A receptor (Larios *et al* 2014). Glutamine formed from GS activity might plays an important role in sensing the plant nitrogen status and regulates the uptake and nitrate reduction (Glass *et al* 2002). According to Masclaux *et al* (2001), it was suggested that significant variations occurred in physiological traits and yield components of maize and also found a positive correlation between nitrate content, GS enzymatic activity and yield. In a study, the analysis of the GS components in wheat leaves showed the change in the balance of GS1 and GS2 activity in the flag leaves (Nemeth *et al* 2018).

Goel and Singh (2015) observed that abiotic stresses such as of low temperature affected the process of ammonia assimilation by changing GS activity in Brassica. Lu *et al* (2005) clarified that there was a relationship between GS activity and proline metabolism under low temperature stress. In a previous study on oats, GS activity was found to be maximum at late vegetative stage as compared to early ones (Kaur and Goyal 2016). In an earlier study, genotype Glu 1356 of wheat showed highest activity at all growth stages among other genotypes (Kaur *et al* 2015). The high GS activity during different growth stages depictedextensive ammonia assimilation and remobilization which are highly interrelated processes at plant growth and development (Balotf *et al* 2015). N efficient genotype of wheat YM49 had increased assimilation ability remobilisation ability at different maturity stages than N-inefficient genotype XN509 (Zhang *et al* 2017). GS activity is an efficient

physiological marker to indicate nutrition conditions and N status in barley (Avine-Ospine *et al* 2015), maize (Prinst and Espen 2015) and wheat (Balotf *et al* 2015). Mrid *et al* (2018) suggested that GS activity was high during initial stages of seed growth in sorghum. GS Genotypic differences were also present in terms of GS activity in oats. Maximum activity was recorded in Kent and minimum in OL-125 (Kaur and Goyal 2016).

2.1.2.2 Glutamate synthase (GOGAT, EC 1.4.1.14)

Glutamate synthase catalyses the reductive transfer of amide group from glutamine to α-ketoglutarate forming two molecules of glutamate (Krapp *et al* 2005). Together with GS, it maintains flow of nitrogen from NH_4^+ into glutamine and glutamate which are then used for several other aminotransferases reactions during the synthesis of amino acids (Nigro *et al* 2013). It occurs as two distinct isoforms which differed in subunit composition, molecular weight, reductant specificity and metabolic function.

- Ferredoxin dependent plastidic isoform (Fd-GOGAT)
- NADH dependent cytosolic isoform (NADH-GOGAT)

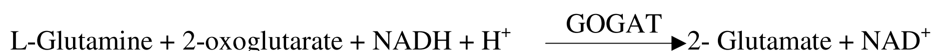
The plastidic isoform of GOGAT are involved in the reassimilation of photorespiratory ammonium in conjunction with GS2 whereas the cytosolic isoform are involved in the synthesis of glutamate mostly in non-photosynthetic tissues to sustain plant growth and development (Hirel and Limami 2004).

Ferredoxin dependent GOGAT: It is an iron-sulphur flavoprotein composed of a single subunit with molecular mass of 140-160 kDa.

$$\text{L-Glutamine} + \text{2-oxoglutarate} + \text{Fd (reduced)} \xrightarrow{\text{GOGAT}} \text{2-Glutamate} + \text{Fd (oxidized)}$$

In C3 plants, it is located in the chloroplast of leaves whereas in C4 plants it is present predominantly in bundle sheath chloroplasts. It mainly accounts for more than 95% of the GOGAT activity in photosynthetic plant tissues (Suzuki and Knaff 2005). The enzyme activity increases with the growth of the plant in the presence of light.

NADH dependent GOGAT: It is also known an iron-sulphur flavoprotein and a monomeric protein with molecular weight of 240 kDa. It is found primarily in non-photosynthetic tissue such as etiolated leaf tissue, seeds, roots and companion cells.



$$\text{L-Glutamine} + \text{2-oxoglutarate} + \text{NADH} + H^+ \xrightarrow{\text{GOGAT}} \text{2- Glutamate} + NAD^+$$

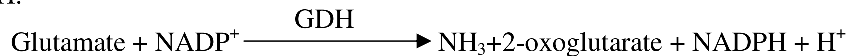
The activity of NADH dependent enzyme was found to be about 2-25 folds lower in green leaves and non-green tissues like seeds and roots in comparison to Fd dependent GOGAT. In particular, NADH-GOGAT can assimilate ammoniafrom both primary and secondary sources during N remobilization while Fd-GOGAT plays a crucial role in the reassimilation of NH_4^+ during photorespiration (Lea and Miflin 2003).

In a previous study on wheat, at tillering and post anthesis stage the activity of GOGAT was very high in comparison to anthesis stage (Kaur *et al* 2015). In addition to this,

maximum GOGAT activity was reported in Glu 1356 as compared to other genotypes of wheat (Kaur *et al* 2015). Gupta *et al* (2019) also reported variable GOGAT activity in different genotypes of Brassica. In an earlier study on oats, minimum GOGAT activity was reported in Kent and maximum in OL-9 (Kaur and Goyal 2016).

2.1.2.3 Glutamate dehydrogenase (GDH. EC 1.4.1.2)

Glutamate dehydrogenase (GDH) catalyses the ammonium incorporation into glutamate by reductive amination of 2-oxoglutarate (McAllister *et al* 2012). Glutamate dehydrogenase is one of the few enzymes capable of releasing amino nitrogen from amino acids to give a keto acid and ammonia that can be separately recycled to be used in respiration and amide formation. GDH catalyses the following reaction by using either NADH or NADPH.



$$\text{Glutamate} + NADP^+ \xrightarrow{GDH} NH_3 + \text{2-oxoglutarate} + NADPH + H^+$$

The enzyme protein exists as hexamer consisting of two subunit polypeptides α and ß having subunit molecular mass of 41-45 kDa. Under normal growth conditions, α subunit homohexamer, exhibits strong deaminating activity and only a very low aminating one (Skopelitis *et al* 2007) while the ß-subunit homohexamer, predominantly deaminates Glu (Purnell and Botella 2007). Plant GDH has a very high km for NH_3 and it is located in the mitochondria of variable tissues of plant. GDH protein is mostly present in the mitochondria of the phloem companion cells (Dubois *et al* 2003). GDH plays an essential role in maintaining glutamate haemostasis. It was suggested in a study most of the available NH_3 in higher plants is assimilated through GS/GOGAT pathway but the rest by GDH activity (Lea and Ireland 1999). Miyashita and Good (2008) claimed that GDH activity is required for the survival of plant during dark conditions. This clearly indicate that GDH pathway plays a complementary role under conditions of ample ammonia supply or adverse environmental conditions (Stitt *et al* 2002, Ferrario-Mery *et al* 2002, Lu *et al* 2005). Some studies suggested that GDH could operate in the direction of NH_3 assimilation but most studies indicate that GDH primarily functions in the direction of glutamate deamination (Lea and Miflin 2003). In a study, it was reported that ammonia might be a signal responsible for the enzyme induction, so the enhanced ammonium content might be responsible for the higher NADH-GDH activity (Ferrario-Mery *et al* 2002). GDH may also function in the direction of glutamate biosynthesis, under conditions of carbon and ammonium excess (Abiko *et al* 2010, Lehmann *et al* 2010). GDH is responsible for maintaining the balance between the metabolic pool of carbon and nitrogen compounds during each developmental phase, from seed germination to seed formation (Miflin and Habash 2002).

Goel and Singh (2015) observed increased GDH activity in response to cold stress. In a previous study on wheat and barley, measurement of GDH activity demonstrated that the aminating (NADH-dependent) enzyme activity is significantly higher in roots than in leaf

tissues (Mahboobi *et al* 2002). During the stress conditions such as high nitrate levels, the activity of GDH initially increase to some extent to prevent the accumulation of ammonia but the activity suddenly decreases (Zhang *et al* 2017). Previous worker suggested that, whatever be the growth conditions or developmental stage of the plant, the variations in Glutamate content are relatively minor in comparison with most of the other amino acids (Forde and Lea 2007).

The GDH activity also differed with respect to growth stages. In a previous study, it was observed that the NADH-dependent GDH aminating enzyme activity increases 2-fold from 14 DAF to 28 DAF in the flowering plants (Kichey *et al* 2005). During ageing of the flag leaf, a similar pattern of expression was obtained when the GDH aminating activity was monitored in wheat. Xiaochuang *et al* (2019) showed differential response of two varieties of rice in response to different growth conditions. It was reported in a study that GDH activity varied among cultivars of wheat (Guellim *et al* 2019).

2.1.3 Transaminases

Transaminases or aminotransferases are a group of enzymes which catalyses the interconversion of amino acids and oxo acids by the transfer of amino groups. Glutamate oxaloacetate transaminase and glutamate pyruvate transaminase are the two transaminases of great significance in plants. These transaminases catalyses the key reactions in the metabolism of glutamate, therefore, the nitrogen present in glutamine and glutamate can be transferred to wide variety of amino acids, nucleic acids (Forde and Lea 2007) and ureides (Amarante *et al* 2006).

Glutamate oxaloacetate transaminase (GOT, EC 2.6.1.1)

Glutamate oxaloacetate transaminase, also known as aspartate aminotransferase is a ubiquitous pyridoxal phosphate dependent enzyme that exists in both mitochondria and cytosolic forms.

Glutamate and aspartate are each required for separate but essential steps in the urea cycle. The free movement of nitrogen between the glutamate and aspartate pools is an important balancing process that is vital for normal cellular metabolism (De la Torre *et al* 2014).

$$\text{L-Aspartate} + \alpha\text{-ketoglutarate} \xrightarrow{\text{GOT}} \text{L-Glutamate} + \text{Oxaloacetate}$$

Glutamate pyruvate transaminase (GPT, EC 2.6.1.2)

Glutamate pyruvate transaminase, also known as alanine aminotransferase catalyses the reversible conversion of L-alanine and α-ketoglutarate to L-glutamate and pyruvate. It hastwo distinct molecular and genetic forms: one cytoplasmic (soluble) (GPT1) and one mitochondrial (GPT2). This enzyme maintains the carbon-nitrogen balance through alanine and pyruvate.

The increased ammonia assimilation capability which controls plant growth and development through inorganic N resulted in changes to the levels of nitrogenous compounds, one of them, glutamate occupies an important position in nitrogen metabolism (Forde and Lea 2007). Glutamate could act as an important signal to modulate root growth through monitoring changes in auxin distribution in plant tissues. In a previous study on wheat, the activities of GPT and GOT increased under increased ammonium conditions (Wang *et al* 2016). Similar results were reported in rice by Mo *et al* (2003).

The activity of GPT and GOT differed with respect to growth stages. In a previous study on wheat, the activity of GPT and GOT was maximum at anthesis stage followed by tillering and post-anthesis stage (Kaur *et al* 2017). Asthir and Tak (2017) suggested that concentration of proteins as well as amino acids may be responsible for enhanced GPT and GOT enzyme activities. Plant response to ammonium concentration varied significantly among cultivars (Li *et al* 2011). Xumai25 genotype of wheat contains higher glutamate content which results in increased activities of GPT and GOT in comparison with AK58 (Wang *et al* 2016). The activity of GPT and GOT varied in response to two different growth stages in wheat and also found variations among six genotypes (Asthir *et al* 2018). Kaur *et al* (2017) reported that the activity of GPT and GOT varied among six wheat genotypes. Asthir and Bhatia (2014) also investigated genotypic variations among three wheat genotype.

2.2 Nutritional composition and digestibility in oat

2.2.1 Dry matter content

Dry matter refers to content remaining after removal of water. The nutrients in feeds, required by the animal for maintenance, growth and lactation, are part of the DM portion of the feed. However, weather and environmental conditions, such as humidity, rain and snow, all affect feed dry matter content. In an earlier study wheat, highest dry matter accumulation was recorded in the crop sown in the November month as compared to December month and also observed differences among genotypes (Alam *et al* 2013). According to Tahir *et al* (2009), early sowing resulted in higher dry matter content in wheat in comparison with late sowing. Similar trend in fodder wheat was observed by Shahzad *et al* (2002).

Filho *et al* (2011) found increased dry matter content with advancing age of the corn forage. Ziki *et al* (2019) also recorded high dry forage weight at 56 DAS as compared to 42 DAS. A nutritional study on oat observed variation in dry matter content among twenty seven genotypes (Wagh *et al* 2019). Another study reported variable dry weight in Kalash, F-414 and CK-1 genotypes of oat (Hameed *et al* 2014).

2.2.2 Crude protein

Oat is considered an essential source of proteins with high nutritive value and

proteins are the essential element of livestock nutrition. Crude protein (CP) has a large impact on animal digestive health and productivity. Crude protein is a chemical analysis of the forage that calculates the amount of nitrogen, which is the building block of amino acid that form proteins. In particular, forages with high concentration of crude protein are needed by livestock to enhance the milk production and for the digestion of the feed components (Eskandari *et al* 2009). Crude protein concentration generally varied by seasonal variations. Cooler environments produced oat forage with higher levels of CP as compared to warmer months. In a study on staggered sowing in oat, the crop sown in the October month recorded highest crude protein content as compared to September month (Jehangir *et al* 2013). In another study, high CP content was recorded in oat sown in the month of November in comparison with October month (Dar *et al* 2014). This might be due to less utilization of the N for higher biomass production because of low temperature during the late sowing.

As forage plants mature, the crude protein content decreases because the plant moved from a vegetative to a mature stage. It appeared that high CP content at early stages of growth was owing to more leafy nature and succulency of crop (Hussain *et al* 1995). One of the recent studies recorded that early fodder cutting at 50 DAS resulted in maximum CP content in barley than the late cutting time (Dhillon *et al* 2019). Lowest CP content was observed in maize at 65 DAS followed by 55 DAS and 45 DAS (Salama 2019). The CP content in forage oat was five fold higher in seedling state than the full grain maturity state (Liu and Mahmood 2015). Flowering stage in oat presented higher CP content as compared to dough stage (David *et al* 2010). Similar results were investigated in oat at different maturity satges (Mobashar *et al* 2018).

Different cultivars of oat also recorded significant difference in crude protein content. In a previous study, Palampur-1 variety of oat recorded higher CP content in comparison with Kent (Sharma *et al* 2019). Similar findings were reported in different varieties of fodder oat (Kumar *et al* 2001). Hameed *et al* (2014) cocluded that variety SK-1 exhibited maximum CP content (9.97%) and it was followed by F-414(9.43%) and Kalash (8.60%) varieties. Dar *et al* (2014) observed that SKO-20 being an early maturing variety has maximum content of 10.51% in comparison with SKO-108 and Sabzar. The protein content of different genotypes of oat grain varied from 9.7% to 17.3% (Ryan *et al* 2007).

2.2.3 Non protein Crude Protein and True protein

Non protein crude protein is a term used in animal nutrition to refer collectively to compounds such as urea, biuret and ammonia which are not proteins but could be a source of nitrogen to ruminants. Non protein crude protein (NPCP) includes all nitrogen sources that are not a part of polypeptide. Non protein crude protein are abundant in fodder crops such as oats. But too high levels cause a depression in growth and possible ammonia toxicity because microbes convert non-protein nitrogen to ammonia first before using that to make protein

(Tadele and Amha 2015). In a previous study, low temperature stress stimulated the accumulation of non protein nitrogen (Liu *et al* 2017).

Ruminal microbes of cattle and sheep can utilize non-protein nitrogen to synthesize proteins that can replace a portion of their total dietary protein requirements. More N is mobilised from older tissue with plant maturity where proteolysis of chloroplast proteins recycles amino acids back into the N pool for new growth (Hortensteiner and Feller 2002). Secondly, sheath material containing greater concentrations of non protein nitrogen relative to lamina was more apparent in older pastures as a result of successive leaf appearance and elongation of sheath above the 5 cm cutting height (Hoekstra *et al* 2008). Non protein crude protein content decreases with maturity of the plant. According to Kaur and Goyal (2017), highest non-protein nitrogen was observed at 30 DAS and lowest at 60 DAS.

True protein represents the nitrogen present in the protein part of the forages. Aghaee *et al* (2011) suggested that poteins play a protective role against the low temperature stress prevailing during the growing period. Additionally, Koc *et al* (2010) clarified that protein synthesis is an essential process to increase tolerance against abiotic stress such as of low temperature. Zhang *et al* (2017) investigated protein-N content in different parts of the plant at different growth stages. Meanwhile, difference in protein content in two wheat genotypes at two different maturity stages was also reported (Abid *et al* 2018).

2.2.4 Free amino acids

Amino acids primarily are the nitrogenous compounds that play a diverse role in metabolism and are majorly transported into phloem (Tilsner *et al* 2005). They are important part of animal's feed and are important for the biosynthesis of glycoproteins, neurotransmitters, polyamines and nitric oxide (Murray 2016). The nitrogen present in glutamine and glutamate can be transferred to various types of amino acids, nucleic acids (Forde and Lea 2007) and ureides (Amarante *et al* 2006). A nutritional study depicted that the amino acid content modulate the gene expression of plants (Themelis *et al* 2017). The contents of free amino acids are very important for the evaluation of protein rich food.

The free amino acid content always depend upon the growth environments such as sunshine, nutrients available and their growth pattern. A recent study on maize claimed that low temperature stress had a remarkable influence on free amino acid content (Zhang *et al* 2020). Similar findings were recorded in oat genotypes (Goyal and Kaur 2018).In a previous study, maximum amino acid content was observed in mature leaves as compared to young and senescent leaves in sunflower (Roy *et al* 2013). Kaur and Goyal (2017) analysed that free amino acids were lowest at 30 DAS (1.98 mg g^{-1}) and highest at 60 DAS (3.85 mg g^{-1}) in oat. Asthir *et al* (2018) observed differential response of wheat genotypes in relation to free amino acids. The amino acid concentration was variable in Antek, Skarb, Nagradowicki and Granal varieties of barley (Biel and Jacyno 2013). It was suggested that when the availability of soil

nitrogen exceeds the plant demand, accumulation of nitrogen occurred in the form of nitrate, amides and free amino acids (Bryant *et al* 2012).

2.2.5 Crude Fat

Crude fat chiefy consists of unsaturated fatty acids, waxes, oil-soluble pigments, free fatty acids, phospholipids and cholesterol. Crude fat content is essential for the livestock as it maintains the structural tissues of the body by acting as the reserve energy. In a recent study on oats, sowing between October and November months leads to variation in the crude fat content from 2.52% to 2.34% (Kadam *et al* 2019). Higher crude fat content with timely sown crop may be due to availability of favourable environment for rapid metabolic activities and better biomass production in comparison to late sown crop (Kumar 2012).

Crude fat content was more affected by season than by precipitation and it was detected that fats were declined with advancing age of vegetative parts of plant that might be due to translocation of fatty material to growing fractions (Earl Willard and Schuster 1973). Hussain *et al* (2009) studied variation in mean values of fats at different stages of harvest in some forage crops.

In a previous study, differential response of four sorghum genotypes was observed for crude fat content (Prajapati *et al* 2017). Several studies reported genotypic differences in different fodder crops in relation to crude fat content (Bial and Jacyno 2013, Anjum *et al* 2014, Biel *et al* 2016, Meena *et al* 2018). In another study, highest crude fat content was recorded in STH 6106 and lowest in Krezus genotype of oat (Biel *et al* 2014).

2.2.6 Ash

Ash content represents the percentage of mineral content present in the forages. Minerals in the feed can be categorized into two types i.e. internal and external. Minerals such as Ca, P, K, Mg etc are normally present in plants and are called internal minerals. These minerals are of high nutritional value for lactating dairy cows. The minerals which are associated with soil such as silica are called external minerals. So forages should contain as little soil contamination as possible (Hoffman 2005). Ash content in oat can have a significant role in animal performance. Ash content is influenced by climatic conditions. Heavy rains during the growth of crop contribute to higher ash values which have the potential to splash soil particles onto forages. According to Kadam *et al* (2019), ash content varied from 13.3% to 12.5% in a staggered sowing between October and November months. The higher ash content due to early sown crop may be due to higher better climatic conditions as compared to delayed sown crop. Similar findings were reported by Kumar (2012) and Jehangir *et al* (2017) in oats. Another researcher also reported variation in ash content with respect to different sowing dates and wheat genotypes (Ahmed and Hassan 2015).

Ash content decreases with maturity because mineral concentration decreases (Pathan *et al* 2012). Similar trend was observed in maize results by Shehzad *et al* (2012). The

variation in the concentration of ash content with advancing maturity was also reported by Kitabi and Tamir (2007). In an earlier study, it was observed that ash percentage varied among different oat genotypes (Khan *et al* 2014) and sorghum genotypes (Singh *et al* 2017). It was due to different efficiencies of genotypes to absorb nutrients depending upon variable rooting depth and rooting pattern. According to Hameed *et al* (2014), ash percentage of 3 oat genotypes named Kalash, F-414 and CK-1 lied in the range from 13.70% to 9.544%.

2.2.7 Nitrate content

Nitrate is a normal constituent of plants and is the main source of nitrogen in the soil. Nitrate content in plant material becomes dangerous when its amount rises and exceeds the amount needed to achieve a sufficient dry matter. Many environmental conditions are responsible for nitrate accumulation in plants such as lack of sunlight, continuous cloudy days, low temperature, hail, frost etc. (Ramteke *et al* 2019). Nitrate toxicity in ruminants mainly occurs with the consumption of cereal crops such as oat, maize, barley etc. An excessive amount of nitrate got accumulated in rumen from where it is readily absorbed into blood stream and combines with ferrous ion of haemoglobin to form methaemoglobin (met-Hb). The met-haemoglobin is poor transporter of oxygen in the body and the animal suffers from oxygen deficiency. Fodder crops with more than 2000 ppm nitrate-N are considered toxic (Kaur and Goyal 2016). In a recent study on barley, it was reported that nitrate content increases with delaying the sowing from October to November due to low temperature prevailing during November month (Dhillon *et al* 2019).

Nitrate content is also greatly affected by plant maturity. The lower level of nitrate-N in mature crops was generally associated with increased enzyme activities or due to reduced uptake of NO_3^- ions by plant (Sidhu *et al* 2011). Nitrate content also varied with plant parts. Flowers and stem has higher nitrate content than leaves which explained the lower nitrate-N with plant ageing (Taute *et al* 2002). Grains does not contain appreciable amount of nitrates (Bolan and Kemp 2003). Radiositis *et al* (2000) concluded that young crop contain more nitrate levels as compared to mature crop. In an earlier study on oat, it was observed that nitrate content declined from vegetative stage to maturity stage (Monica *et al* 2018). In another study, it was reported that nitrate content was maximum in fodder triticale during stem elongation and it furthers declined at booting and maturity stage (Gulmezoglu *et al* 2010).

Nitrate content also varied among different genotypes. In an earlier study, different oat genotypes were studied for nitrate content and highest was recorded in Kent (2062 ppm) followed by OL-9 (2059 ppm), OL-125 (2031 ppm) and OL-10 (1970 ppm) (Kaur and Goyal 2016). Monica *et al* (2018) also reported that nitrate content was highest in HFO865 genotype and lowest in Dunav-1 genotype of oat.

2.2.8 Nitrite content

Nitrite found in forages are formed from nitrate, which is normally converted to ammonia then to amino acid and finally to protein. Nitrite usually do not accumulate in plants and was found in very low concentration as compared to nitrate (Hunt and Turner 1994). Sometimes the rate of nitrate reduction was much faster than nitrite reduction. In that case, nitrite started accumulating in plants. Nitrite is ten times as toxic to livestock as nitrate and nitrite content had a remarkable effect on the activity of nitrogen metabolism enzymes. Ali *et al* (2007) stated that nitrite alongwith nitrate inhibited the activity of enzyme nitrate reductase in rice. They also explained that in the presence of light, nitrite partially increased the nitrate induced nitrite reductase activity but in dark, it inhibited its activity. Uwah *et al* (2009) found accumulation of nitrite content among green leafy vegetables. On the other hand, Shokrzadeh *et al* (2007) recorded very low concentrations of nitrite content in spinach leaves.

2.2.9 Cell wall constituents (ADF and NDF)

Acid detergent fiber (ADF) and neutral detergent fiber (NDF) are important parameters used in forage food consumed by animals. Neutral detergent fiber is the insoluble portion of the forage which represents cellulose, hemicellulose, lignin and silica content commonly referred to as cell wall fractions. The level of NDF in forages influences the ruminant's intake potential of dry matter as well as the time of rumination. NDF is negatively associated with energy concentration in ruminant feed. As the NDF content in forages increased, animals would consume less forage (Eskandari *et al* 2009). The process of estimating NDF content includes a neutral detergent solution that dissolves sugars, plant pectins, lipids and proteins thus leaving behind the fibrous part. Higher content of NDF leads to lesser energy, milk production as well as dry matter digestibililty in ruminants. ADF is that portion of forage that contains the components which are least digestible by livestock including cellulose, lignin and silica content. ADF is important because it is negatively correlated with digestibility of feed and food. As the ADF increases, the digestibility of forage decreases because lignin and silica are indigestible in ruminants (Bao *et al* 2007). Many authors found synergistic association between ADF and NDF in fodder crops (Cardinal *et al* 2003, Krakowsky *et al* 2005 and Grandlo *et al* 2005).

The NDF and ADF content changes over growing season due to weather affecting the balance between photosynthesis and temperature. During cool weather conditions, when the crop is in the vegetative state, the NDF content remained low. ADF and NDF content varied in spring and autumn sown oats due to difference in temperature (Aydin *et al* 2010). Many nutritional studies were done regarding the ADF and NDF content (Sasani *et al* 2004, Jafarian *et al* 2016 and Soni *et al* 2016). NDF and ADF content increases with stages of harvest of crop which might be due to more stiffness of cell wall as well as more structural carbohydrates synthesis (Thakare *et al* 1978). Albayrak and Yuksel (2010) also found

enhanced ADF and NDF content with advancing age of fodder beet.

According to Mut *et al* (2016), ADF and NDF content varied in 25 genotypes of oats and also claimed that the variation in NDF content among variable genotypes may be due to changes in growth pattern and formation of structural biomass with advancing age of plant.

2.2.10 *In vitro* dry matter digestibility

Digestibility mainly refers to the extent to which forage is absorbed as it passes through an animal's digestive system**.** *In vitro* dry matter digestibility determines the nutritional value of ruminant feeds. The chemical linkages between lignin and hemicellulose are the principal plant factors restricting digestion of the plant cell wall by ruminants. Aprevious research on oats suggested higher digestibility of fresh forages in comparison with hay and silage (Colabro *et al* 2005). A number of nutritional studies were done to evaluate the*in vitro* dry matter digestibility in different fodder crops (Datt *et al* 2006, Datt *et al* 2009 and Gate *et al* 2018). Differences in climatic conditions influences digestibility of forages. In a previous study on barley, late sowing in the month of November recorded higher digestibility than timely sowing in the month of October (Dhillon *et al* 2019).

IVDMD content generally declined with advancing age of the fodder crops which must be due to lignification of the cell wall (Bora *et al* 2011). Therefore, the cell wall fractions may have a negative influence on forage digestibility. In an earlier study, IVDMD content in oat decreased with delay in harvesting from 50 to 70 DAS (Malik *et al* 2015). This might be due to the fact that, at the later stage the stem comprise a much larger portion of the plant than leaves (Mcdonald *et al* 2001). Kaur *et al* (2013) concluded that barley harvested at 45 DAS had higher DM digestibility (79.94%) in comparison with 60 DAS (76.06%).

According to Kafilzadeh and Heidary (2013), the IVDMD content varied in 18 different genotypes of oat. Genotypic differences were also reported in maize (Vaswani *et al* 2015). Such a variation in IVDMD content was because of combined effect of CP content, ash content and cell wall fractions. In another study on oats, Monica *et al* (2018) reported that maximum IVDMD content was observed in HFO498 (80.6%) and minimum in Kent (58.56%).

2.2.11 Chlorophyll content

Chlorophyll is the pigment that provides the plant its characteristic green colour. Chlorophyll content plays a vital role in the physiology, productivity and economy of fodders. The amount of chlorophyll present in the leaves is an indicator of photosynthetic activity of plants. Chlorophyll is highly influenced by nutrient availability and environmental stresses such as cold, salinity etc. In general, higher plants contain two forms of chlorophyll i.e. chlorophyll a and chlorophyll b. The two forms predominantly differed in their coloured content, chlorophyll a is bluish green while chlorophyll b is yellowish green. Usually, the concentration of chlorophyll a is three times higher as compared to chlorophyll b in leaves.

Chlorophyll content might be indicative trait for characterizing how the fodder crops respond to climate change. The enzymatic reactions for the synthesis of chlorophyll are regulated by temperature (Nagata *et al* 2007). The optimum temperature in general for chlorophyll synthesis is 30°C (Nagata *et al* 2005). Temperature higher or lower than optimum affects the original chlorophyll content. Precipitation might affect the photochemical activity of chloroplast (Zhou 2003). N is the structural element of chlorophyll, thereby affects formation of chloroplasts and accumulation of chlorophyll in them (Tucker 2004). Mobilisation of chloroplast N has a central role in leaf lamina metabolic activity and canopy (Hortensteiner and Feller 2002).

Chlorophyll content decreases with plant maturity (Kaur *et al* 2015). Leaf thickness is one of the factors that determine chlorophyll content under different growth conditions, varieties and growth stages. Leaf thickness changes according to age and environment of the plant (Gratani and Bombelli 2000). In a previous study, maximum chlorophyll content was recorded in OS 377 genotype of oats followed by OS 6 and HJ-8 (Devi *et al* 2019). Similar results were reported in wheat by Karele (2001).

2.3 Yield and yield components in oat

2.3.1 Plant height

Plant height is a major contributor towards forage yield of crops (Ahmad *et al* 2012). Different planting dates significantly affected the plant height of oat varieties due to variation in genetic and environmental factors (Jehangir *et al* 2013). The higher temperature available to the early sown crop in terms of plant height produced more green fodder yield (Sharma and Verma 2007). Dar *et al* (2014) observed that plant height declined with late planting from October to November. This may be attributed to longer growth period and better conditions available during optimum sowing dates. Plant height varied from 73.76 cm to 65.12 cm with the delay in planting date (Tahir *et al* 2009). Early sown crop may have enjoyed the better environmental conditions especially the temperature and solar radiation which resulted in tallest plants (Shahzad *et al* 2002). Different varieties of fodder crops affected the plant height significantly (Ayub *et al* 2011). Genotype Sehar-06 showed maximum average plant height (101 cm) as compared to other varieties of wheat (Mumtaz *et al* 2015). Among different varieties of fodder oats, Sabzar recorded higher plant height than SKO-20 and SKO-108 (Dar *et al* 2014). High yielding varieties tends towards more plant height (Naeem *et al* 2006).

2.3.2 Leaf length and Leaf breadth

Leaf length and breadth is a key factors determining the fodder yield of crops (Humphreys 2005). Leaf length has strong response to environmental conditions such as temperature, nitrogen availability, moisture conditions, defoliation frequency and availability of light. One of the major response to light competition in plants is an increase in plant height i.e. leaf length and breadth during the vegetative growth of forages. Leaf length and breadth

plays a great role in shaping the physiological structure of the canopy. Leaf length in oat is greatly influenced by the maturity stages of the plant i.e. both vegetative and reproductive stages of growth. The change in leaf growth rate might be due to an increase in cell division as the length of the leaf is mainly determined by its constituent cells and their length. In an earlier study, leaf length varied from 122.2-140.9 cm and leaf breath varied from 2.4-3.5 cm in oat genotypes (Kaur and Goyal 2017). Similar results were observed in oat varieties by Dubey *et al* (2013).

2.3.3 Number of tillers

Tillers are an important component for oat growth and regrowth. Fageria *et al* (2006) stated that the maximum efficiency of tillering is examined by the supply of photoassimilates and mineral nutrients. In a previous study, the maximum number of tillers were observed during optimum planting time as compared to delayed planting in wheat (Mumtaz *et al* 2015). This might be due to long growing period, maximum nutrients absorbing from the soil and light available from the sun to optimum sown crop (Seleiman *et al* 2011). Similar findings were reported in wheat by Shah *et al* (2006), Kalwar *et al* (2018). Number of tillers in oats increased with palnt maturity (Khan *et al* 2014). Ayub *et al* (2011) observed differences among oat genotypes in relation to number of tillers. In a previous study, maximum number of tillers was observed in Kent followed by JHO-822 and RO-19 (Singh *et al* 2018). Similar findings were reported by Waseem-ul-Hassan *et al* (2000) and Hameed *et al* (2014).

2.3.4 Number of leaves per plant

Number of leaves per plant is an essential component of forage quality as it plays a vital role in growth and development of plants. Gasim (2001) concluded that increased number of leaves per plant could be attributed due to the fact that nitrogen often increased plant height and this resulted in more nodes and internodes and subsequently more production of leaves. The variation in number of leaves per plant had a direct effect on fodder yield of oats (Naeem *et al* 2005). According to Amanullah *et al* (2013), number of leaves per plant differed in relation to growth stages. In the previous study, the number of leaves per plant varied from 7.5 to 5.93 in oat genotypes (Khan *et al* 2014).

2.3.5 Green fodder yield, dry matter yield and crude protein yield

Yield represents the reflection of a plant's potential to accumulate dry matter (Miron *et al* 2007). The yield of the fodders depends upon the time needed for germination as well as better growth of plants as expressed in terms of fresh and dry weight, plant height and number of leaves etc. Optimum date of planting of oat leads to maximum yield of good quality green fodder. Delay in planting decreased the fodder production efficiency of oats as well as decreased the green fodder yield and dry matter yield (Sharma *et al* 2017). It might be due to favourable climatic conditions that have led to luxuriant vegetative growth in the form of leaf stem ratio, plant height, number of tillers and dry matter accumulation (Sheoran *et al* 2017).

Similar findings were recorded by Kumar (2012), Shekare and Lohithaswa (2012), Dar *et al* (2014). According to Atis and Akar (2018), protein yield ranged from 0.60-0.74 t/ha from early to normal sowing of wheat.

The time of harvest also influenced green fodder and dry matter yield and trend of both parameters were exactly similar (Ayub *et al* 2009). According to Kadam *et al* (2019), oats cut at 70 DAS (37.8 t ha^{-1}) recorded maximum green fodder yield as compared to 60 DAS (34.6 t ha^{-1}) and 50 DAS (31.4 t ha^{-1}). Similar pattern was observed for dry matter yield. It might be due to longer vegetative growth available that increased growth and yield attributes and accumulated more photosynthates and dry matter which results in higher fodder production of oats in comparison with earlier sowing (Patel *et al* 2013). Other reports of Malik (2014), Kumar *et al* (2017), Hussain *et al* (2004) also concluded that stage of harvest had remarkable effect on green fodder yield and dry matter yield. Alipatra et al (2013) studied crude protein yield in fodder oat with respect to different cutting management.

Genotypic differences in oats were also present in terms of green fodder yield and dry matter yield (Hameed *et al* 2014). In an earlier study, maximum dry matter yield was observed in JHO-822 followed by Kent and RO-19 (Singh *et al* 2018). In another study, SKO-108 variety recorded maximum green fodder yield over Sabzar and SKO-20 (Dar *et al* 2014). The superiority SKO-108 might be due to thick stem, broad leaf, more vigour and longer growth duration.

CHAPTER III

MATERIALS AND METHODS

The current investigation was conducted on two oat (*Avena sativa* L.) genotypes (OL-10 and OL-11) to study the influence of different planting dates (9th Oct, 24th Oct, 13th Nov, 26th Nov, 11th Dec and 26th Dec) and growth stages (40, 50 and 60 DAS). The crop was raised in the field of Forages, Millet and Nutrition section and experiments were carried out at Forage Evaluation Laboratory, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The materials and methods employed in the present research have been mentioned under following headings and sub-headings:

3.1 Raising of oat (*Avena sativa* L.) crop

3.2 Collection of fresh leaf samples

3.3 Chlorophyll content

3.4 Extraction of Nitrite reductase, Glutamate synthase and Glutamate dehydrogenase, Glutamate oxaloacetate transaminase and Glutamate pyruvate transaminase

3.5 Enzyme assay

- 3.5.1 Nitrate reductase (EC 1.6.6.1, NR)
- 3.5.2 Nitrite reductase (EC 1.7.7.1, NiR)
- 3.5.3 Glutamine synthetase (EC 6.3.1.2, GS)
- 3.5.4 Glutamate synthase (EC 1.4.1.14, GOGAT)
- 3.5.5 Glutamate dehydrogenase (EC 1.4.1.2, GDH)
- 3.5.6 Glutamate oxaloacetate transaminase (EC 2.6.1.1, GOT)
- 3.5.7 Glutamate pyruvate transaminase (EC 2.6.1.2, GPT)

3.6 Collection and storage of oat samples

3.7 Nutritional composition and digestibility of oat samples

- 3.7.1 Dry matter content
- 3.7.2 Crude protein
- 3.7.3 True protein and non-protein crude protein
- 3.7.4 Free amino acids
- 3.7.5 Crude fat
- 3.7.6 Ash
- 3.7.7 Acid detergent fiber
- 3.7.8 Neutral detergent fiber
- 3.7.9 *In vitro* dry matter digestibility

3.8 Antinutritional composition of oat samples

- 3.8.1 Nitrate-N
- 3.8.2 Nitrite-N

3.9 Yield and yield parameters

3.9.1 Leaf length

3.9.2 Leaf breadth

3.9.3 Plant height

3.9.4 Tillers sq m^{-1}

3.9.5 Number of leaves per plant

3.9.6 Green Fodder yield

3.9.7 Dry Matter yield

3.9.8 Crude Protein yield

3.10 Statistical analysis

3.1 Raising of oat (*Avena sativa* L.) crop

Oat crop was raised at Forage Experimental Farm during *Rabi* season of 2019. The treatment combinations comprised of six planting dates that were characterized as optimum planting date (Oct 24), sub-optimum planting dates (Oct 9 and Nov 13) and late planting dates (Nov 26, Dec 11 and Dec 26). During experimentation, highest mean temperature and sunshine hours were recorded in October month and lowest in December month. The mean temperature and sunshine hours ranged from 9.6-25.8°C and 0.5-7 hrs. The crop was planted in plots measuring 4.0 meter x 2.5 meter using randomized block design (RBD) in 3 replications. Each plot comprised of 10 rows with row to row spacing of 25 cm and fertilizers were applied in the form of urea as per treatment with full dose of nitrogen at the time of sowing. Salient features of the two studied genotypes is mentioned as follows:

Genotype	Year of release	Salient features
OL-10	2014	Multicut variety and on an average, it yields about 275 quintals of green fodder per acre.
OL-11	2017	Single cut variety and on an average, it yields about 245 quintals of green fodder per acre.

3.2 Collection of fresh leaf samples

Fresh leaf tissue was used for enzyme assay and chlorophyll content. Second leaf was excised from the plant during morning time (about 9 am) at respective growth stages (40, 50 and 60 DAS). The mid portion of leaf tissue was used for enzyme assay.

3.3 Chlorophyll Content (Barnes *et al* 1992)

0.1 g leaf sample was taken and dipped in 3 ml Dimethyl Sulphoxide solution. The solution was kept in water bath for 1 hour at 60-70°C for colour development. Absorbance was read at 645 nm and 663 nm.

$$\text{Total Chlorophyll} = \frac{20.2 \times A_{645} + 8.02 \times A_{663} \times V}{a \times 1000 \times W}$$

Where,

W = Fresh weight of sample in gram

V = Volume of extract

a = Path length of light in the cell (1 cm)

A_{645} and A_{663} are optical densities

Chlorophyll concentration was expressed as mg/g fresh weight of tissue.

3.4 Extraction of Nitrite reductase, Glutamine synthetase, Glutamate synthase, Glutamate dehydrogenase, Glutamate oxaloacetate transaminase and Glutamate pyruvate transaminase

Reagents:

i) Tris buffer (25 mM, pH 7.5)

ii) 2- Mercaptoethanol

iii) 4.0 mM Magnesium sulphate

iv) 0.25 mM EDTA

Extraction- 0.5 g of fresh leaf sample were extracted in 5 ml of Tris buffer (25mM, pH 7.5) containing 2-mercaptoethanol (2-3 drops), $MgSO_4$ (4.0 mM) , EDTA (0.25 mm). The contents were centrifuged at 4°C for about ten minutes at 10,000 rpm and the filtrate so obtained was used for enzyme assays.

3.5 Enzyme assay

3.5.1 Nitrate Reductase (EC 1.6.6.1, NR) (Hageman and Hucklesby 1971)

a) Reagents

i) **Assay buffer: 0.1M Sodium phosphate buffer (pH 7.5)**-Dissolve 0.1 M Dibasic sodium phosphatein 0.1 M monobasic sodium phosphate.

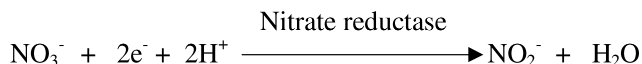
ii) **1% n-Propanol**

iii) **0.25 M Potassium nitrate**

iv) **1% (w/v) Sulfanilamide ($C_6H_8N_2O_2S$) in 1N HCl:** Dissolve 1 g sulfanilamide in 100 ml of hydrochloric acid (1 N).

v) **0.02% N-(1-napthyl) Ethylene Diamine Dihydrochloride (NEDD)**

b) Reaction



$$NO_3^- + 2e^- + 2H^+ \xrightarrow{\text{Nitrate reductase}} NO_2^- + H_2O$$

c) Assay

0.2 g of leaf sample was macerated in 5 ml of assay buffer, 0.25 ml of potassium nitrate (0.25 M) and 0.05 ml of n-propanol. The vials were sealed and incubated in water bath for 90 minutes in dark at 30°C. The nitrite ions (NO_2^-) released into the medium were estimated by taking 1 ml of aliquot and treating it with 1 ml of $C_6H_8N_2O_2S$ (1%) in 1N

hydrochloric acid and 1 ml of NEDD (0.02%). The tubes were kept for 20 minutes and the final vol. was made to 10 ml using distilled water (dw). The OD was noted using Spectrophotometer at 540 nm. The standard curve was made with $NaNO_2$ (0-10 µg) and enzyme activity was expressed as µmol NO_2^- formed h^{-1} g^{-1} FW tissue.

3.5.2 Nitrite reductase (EC 1.7.7.1, NiR)

NiR was assayed as described by Ramirez *et al* (1966) with slight modifications.

a) Reagents

Assay buffer consists of Tris HCl (200 mM, pH 8.0) containing methyl viologen (20 mM) and $NaNO_2$ (6 mM).

b) Reaction

$$NO_2^- + 8H^+ + 6e^- \xrightarrow{\text{Nitrite reductase}} NH_4^+ + 2H_2O$$

c) Assay

0.1 ml of enzyme extract was treated with 0.5 ml of Tris HCl (200 mM, pH 8.0) buffer containing methyl viologen (20 mM) and $NaNO_2$ (6 mM) and diluted upto 0.9 ml using dw. Thereafter, 0.1 ml of sodium dithionite was added to the tubes and incubated at 40°C for 1 hour. After incubation, vortexed the contents vigorously to oxidize the excess dithionite. The vol. was made upto 3.0 ml with dw after taking appropriate amount of aliquot. The quantity of NO_2^- ions in the reaction mixture were determined by adding 1.0 ml ofboth $C_6H_8N_2O_2S$ (1%) in 1N hydrochloric acid and NEDD (0.02%). The tubes were kept for 10 minutes at room temperature and the absorbance was read at 540 nm. The amount of nitrite formed was estimated with $NaNO_2$(0-10 µg) as the standard and the enzyme activity was expressed as µg of NO_2^- removed h^{-1} g^{-1} FW tissue.

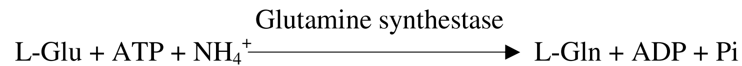
3.5.3 Glutamine synthetase (EC 6.3.1.2, GS)

Glutamine synthetase was assayed as described by Lea *et al* (1990) with slight modifications.

a) Reagents

i) Assay buffer: 0.2 M Tris HCl Buffer (pH 7.5)
ii) 0.05 M Adenosine triphosphate (pH 7.0)
iii) 0.5 M Sodium glutamate
iv) 1.5 M Magnesium sulphate
v) 1 M Hydroxylamine hydrochloride (pH 7.5)
vi) 0.37 M Ferric chloride reagent
vii) 0.5 N Hydrochloric acid
viii) 0.2 M Trichloroacetic acid

b) Reaction



c) Assay:

An appropriate enzyme extract was added to 1 ml of assay buffer, 1 ml of Sodium glutamate (0.5 M), 0.5 ml of adenosine triphosphate (0.05 M, pH 7.0), 0.1 ml of hydroxylamine hydrochloride (1 M, pH 7.5) (freshly prepared) and 0.1 ml of $MgSO_4$ (1.5 M). The vol. of the reaction mixture was made upto 3 ml using distilled water. A blank tube was run simultaneously in which the substrate was omitted. The reaction mixture was incubated for 30 minutes at 30°C and the γ-glutamyl hydroxamate formed was estimated with the addition of 0.5 ml of ferric chloride reagent. The tubes were centrifuged for seven minutes at 12000 rpm and OD was noted using spectrophotometer at 540 nm. The standard curve was made with 0.4-2.0 µM of γ-glutamyl hydroxamate as the standard and the enzyme activity was expressed as µmol γ-glutamyl hydroxamate formed min^{-1} g^{-1} FW.

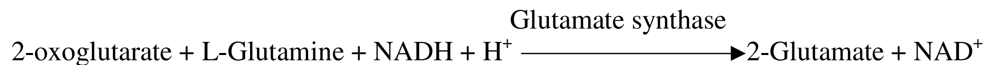
3.5.4 Glutamate synthase (EC 1.4.1.14, GOGAT)

Glutamate synthase was assayed as described by Hecht *et al* (1988) with slight modifications.

a) Reagents:

i) Assay buffer: Tris HCl buffer (0.2 M, pH 8.3)
ii) 2-oxoglutarate (300mM)
iii) Glutamine (300mM)
iv) NADH (4.2 mM)

b) Reaction



c) Assay:

An appropriate enzyme extract was added to 2.5 ml of assay buffer, 0.1 ml of 2-oxoglutarate (300 mM), 0.1 ml of glutamine (300 mM) and 0.1 ml of NADH (4.2 mM). The GOGAT activity was noted at 340 nm for 3 minutes with the decrease in OD after every 15 seconds and was expressed as µmol NADH (oxidized) min^{-1} g^{-1} FW tissue.

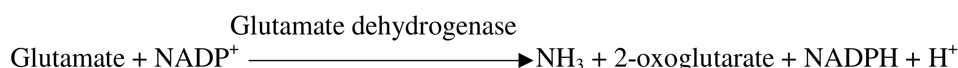
3.5.5 Glutamate dehydrogenase (EC 1.4.1.2, GDH)

GDH was assayed as described by Lea *et al* (1990) with slight modifications.

a) Reagents

i) Assay buffer: Tris HCl buffer (0.2 M, pH 8.3)
ii) 2-oxoglutarate (300 mM)
iii) Ammonium sulphate (300 mM)
iv) NADH (4.2 mM)

b) Reaction



$$\text{Glutamate} + NADP^+ \xrightarrow{\text{Glutamate dehydrogenase}} NH_3 + \text{2-oxoglutarate} + NADPH + H^+$$

c) Assay:

An appropriate enzyme extract was added to 2.5ml of assay buffer, 0.1 ml of 2-oxoglutarate (300 mM), 0.1 ml of NH_4OH (300mM) and 0.1 ml of NADH (4.2 mM). The GDH activity was noted at 340 nm for 3 minutes with the decrease in OD after every 15 sec and was expressed as µmol NADH (oxidized) min^{-1} g^{-1} FW tissue.

3.5.6 Glutamate oxaloacetate transaminase (EC 2.6.1.1, GOT) (Tonhazy 1960a)

a) Reagents

i) Buffered aspartate solution: Dissolve L-Aspartate (0.1 M) in Potassium phosphate buffer (0.1 M, pH 7.5) and the pH was adjusted to 7.0 using KOH (0.5 N).

ii) 2-oxaloglutarate solution (pH 7.0): Prepared by dissolving 0.1 M 2-oxaloglutarate and before making the final volume, the pH was adjusted with KOH (0.5 N).

iii) Pyridoxal phosphate (2 mM)

iv) Trichloroacetic acid (TCA) solution (0.2 M)

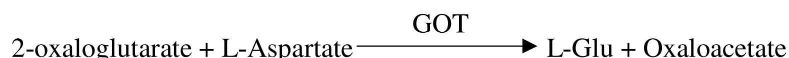
v) Aniline-citrate: Dissolve 5 g of citric acid monohydrate in 5 ml distilled water and aniline (5 ml) was added immediately before use.

vi) 2,4–dinitrophenylhydrazone (5 mM): Dissolve 2,4- dinitrophenylhydrazone (5 mM) in 20% Conc. Hydrochloric acid.

vii) Water saturated Toluene: Dissolve 50 ml of toluene in 10 ml of distilled water and shake it vigorously in separating funnel. The mixture was separated into two phases, the upper layer was filtered using filter paper (Whatman No.1) and the lower layer was discarded.

viii) Alcoholic potassium hydroxide (0.45 M): Dissolve 0.45 M potassium hydroxide in ethanol (95%).

b) Reaction:



$$\text{2-oxaloglutarate} + \text{L-Aspartate} \xrightarrow{\text{GOT}} \text{L-Glu} + \text{Oxaloacetate}$$

c) Assay:

An appropriate enzyme extract was added to 0.2 ml 2-oxaloglutarate solution, 0.5 ml buffered aspartate solution and 0.1 ml pyridoxal phosphate and the mixture was incubated for 30 minutes at 37ºC. The reaction was terminated with the addition of 0.1 ml TCA solution and the contents of the tubes were vortexed vigorously. After the addition of 0.2 ml aniline-citrate, the tubes were shaken and incubated for ten minutes at room temperature. Pyruvate formed, alongwith 2-oxaloglutarate was converted to hydrazine after adding 1 ml of 2,4-dinitrophenylhydrazine solution. Tubes were again thoroughly mixed and allowed to stand for five minutes. After the addition of 2 ml of water saturated toluene, centrifuged the contents

for five minutes at 3000 x g. About 1 ml of the upper layer developed was drawn into another test tube and to this 5 ml of alcoholic potassium hydroxide was added. The absorbance was noted at 520 nm and the standard curve was made using oxaloacetate (0.3-1.8 µmol) as standard and GOT activity was expressed as µmol min^{-1} g^{-1} FW.

3.5.7 Glutamate pyruvate transaminase (EC 2.6.1.2, GPT) (Tonhazy 1960b)

a) Reagents:

Buffered L-alanine solution: Dissolve L-alanine (0.8M) in Tris HCl (0.1 M, pH 7.5) buffer.

The reagents were similar to that of GOT except buffered alanine solution was used in place of buffered aspartate solution. Aniline citrate solution was omitted.

b) Reaction:

$$\text{2- Oxaloglutarate + L-Alanine} \xrightarrow{\text{GPT}} \text{L-Glutamate + Pyruvate}$$

c) Assay:

The assay of GPT activity was similar to that of GOT except aniline-citrate solution step was omitted. The intensity of colour developed was read at 520 nm and the standard curve was made using pyruvate (0.3-1.8 µmol) as standard. The GPT activity was expressed as µmol min^{-1} g^{-1} FW.

3.6 Collection and storage of oat samples

Plant samples (leaves and stem) were collected at certain growth stage (40, 50 and 60 DAS) that were oven dried at 100°C till a constant weight was reached. Thereafter, the dried samples were ground and used for the estimation of nutritional composition, digestibility and antinutritional composition of oat samples.

3.7 Nutritional composition and digestibility of oat samples

3.7.1 Dry matter content (DM)

A known amount of the sample was taken in an aluminium tray and then it was oven dried at 100°C till a constant weight was obtained. The weight of the dried sample was recorded as the DM content of the sample. DM was expressed in percent and calculated by applying the formula:

$$\text{DM content} = \frac{\text{Final dry weight of the sample}}{\text{Initial fresh weight of the sample}} \times 100$$

3.7.2 Crude protein (AOAC 2005)

a) Principle:

Sample is oxidised in the presence of digestion mixture with sulphuric acid to form ammonia (NH_3), CO_2 and H_2O. The NH_3 is trapped in the form of ammonium sulphate $(NH_4)_2SO_4$ in the sulphuric acid (H_2SO_4) solution. Then the known volume of $(NH_4)_2SO_4$ solution formed is steam distilled with NaOH to liberate NH_3 which is absorbed in excess of boric acid. The quantity of NH_3 absorbed to boric acid is estimated by titrating it with standard H_2SO_4.

b) Reagents:

i) Digestion mixture: Prepared by adding copper sulphate ($CuSO_4$) and potassium sulphate (K_2SO_4) in the ratio 1:9.

ii) Sodium hydroxide (40%): 40 g sodium hydroxide was dissolved to make vol. 100 ml with dw.

iii) Indicator: Dissolve 0.3 g methyl red and 0.2 g bromocresol green in 90% ethanol to make volume 400 ml.

iv) Boric acid (4%): Dissolve 4g of boric acid to make volume 100 ml with dw.

v) 0.1N H_2SO_4

c) Estimation:

0.5 g of dried plant sample and 2-3 gof digestion mixture was addedto the Kjeldahl flask. The sample was treated with 10 ml of conc. H_2SO_4 in automated Key Plus digestion. The digestion was done for 2-3 hoursat 450°C until the solution become clear bluish green. Sodium hydroxide solution (40%) was added to the digested sample to create a strong alkaline pHby neutralizing the acid. At the other end, 250 ml flask having 25 ml boric acid (4%) and mixed indicator was placed and it was connected to a two-way condenser. The process of distillation was continued till the volume of distillate became almost doubled. The titration of the distillate was carried out against standard H_2SO_4 (0.1N) until permanent light pink appeared as the end point. Percent nitrogen was determined from the following formula:

$$\% \text{ Nitrogen} = \frac{\text{N} \times \text{vol. of } H_2SO_4 \text{ (0.1N) used} \times 14 \times \text{dilution factor}}{1000 \times \text{wt. of sample (g)}} \times 100$$

Crude protein was determined by applying the formula:

Crude protein = % Nitrogen x 6.25

3.7.3 True proteinand Non-protein crude protein (AOAC 2005)

Estimation:

0.5 g of dry sample was weighed crushed in pestle and mortar with 10 ml of dw. Then 10 ml of 10% trichloroacetic acid (TCA) was added and it was kept overnight. Thereafter samples were filtered on whatman paper 541 and were washed twice with 10% TCA. Then this paper was transferred to Kjeldahl flask and true protein was determined. Non-protein CP was calculated by subtracting true protein from crude protein.

Non-protein CP = Crude protein –True protein

3.7.4 Free amino acids

a) Extraction (Singh *et al* 1978)

0.1 g of dried plant samples were macerated twice with 5 ml of sodium hydroxide (0.1 N) with continuous stirring for 30 minutes and centrifuged for 15 minutes at 14,000 rpm. The supernatant so obtained was filtered and about 2 ml of it was added to 2 ml chilled trichloroacetic acid (20%) and thoroughly mixed. The tubes were incubated at 4°C for 1 h and

the contents were centrifuged for 15 minutes at 14,000 rpm. The contents were filtered and the supernatant so obtained was used for amino acid estimation.

b) Reagents:

Reagent A: Dissolve ninhydrin (1%) in 0.5 M citrate buffer having pH 5.5.

Reagent B: Glycerol (Pure)

Reagent C: Citrate buffer (0.5M)

Ninhydrin reagent: Reagents A: B: C (5:12:2).

c) Estimation (Lee and Takahashi 1966)

Reaction mixture consisted of 0.2 ml of the extract and 5 ml of ninhydrin reagent. The tubes were placed in boiling water bath for 12 min and were cooled at room temperature. Theintensity of colour developed was noted at 570 nm and the standard curve was prepared by using 0.03-0.24 µM of L-gylcine to determine the amount of free amino acids.

3.7.5 Crude fat (AOAC 2005)

a) Principle:

The moisture free sample is extracted in a volatile solvent. The solvent is continuously volatilized, condensed and passed through the sample for extracting soluble components. The solvent is recovered after the completion of the process and the remaining crude fat is made solvent free and weighed.

b) Reagents: The solvent used was petroleum ether.

c) Estimation:

Soxhlet apparatus was used for the estimation of crude fat. 1.0 g of moisture free sample was weighed. It was taken in a thimble having porosity for the rapid passage of solvent. The solvent (60-80°C) was added in the flasks and apparatus was attached to the condenser. The condenser was attached to tap for the circulation of cold water and the extraction was done for eight hrs. The remaining solvent left in the flask after complete extraction was transferred to the 100 ml tarred beaker and was evaporated and the dried residue was cooled and weight was recorded. Crude fat was determined from the following formula:

$$\text{Crude fat} = \frac{\text{Weight of Extract (g)}}{\text{Weight of sample (g)}} \times 100$$

3.7.6 Ash (AOAC 2005)

Estimation:

5.0 g sample was taken in tarred silica crucibles and first incinerated on hot plate till no more smoke comes out. It was then placed in a muffle furnace for 5 hrs at 550-600°C followed by cooling in the dessicator and then weight was recorded. The difference between the initial and final weight of crucible was taken and the ash content was determined from the following formula:

$$\text{Ash (\%)} = \frac{\text{Wt. of residue (g)}}{\text{Wt. of sample (g)}} \times 100$$

3.7.7 Acid detergent fibre (ADF) (Van Soest *et al* 1991)

a) Principle:

The samples are heated in acid detergent solution that dissolves the cell content and hemicelluloses. The remaining residue is known as ADF which consists of lignin, silica and cellulose.

b) Reagents:

Cetyltrimethyl Ammonium Bromide (CTAB)	20.0 g
1 N Sulphuric acid	upto 1 L

Acid detergent solution (ADS)

20.0 g CTAB was mixed in 500 ml of H_2SO_4 (1 N) and volume was made to 1 L with 1 N H_2SO_4.

c) Estimation:

An empty weighed crucible was taken and 1.0 g of sample was added to it. Crucibles were fitted to fibre estimation system (Fibra Plus). Thereafter, about 100 ml of ADS was added, the initial temperature of the instrument was fixed at 500°C and then reduced to 250-300°C when the boiling of the samples had just started. The samples were heated for 1 hr, the remaining solution was drained and the samples were washed thoroughly with distilled water. The crucibles were oven dried and cooled in a desiccator and weighed. Acid detergent fibre was expressed in percent and was calculated by applying the formula given below:

$$\text{Acid detergent fibre} = \frac{\text{(Weight of crucible + fibre) - (Weight of crucible)}}{\text{Weight of sample (g)}} \times 100$$

3.7.8 Neutral detergent fibre (NDF) (Van Soest *et al* 1991)

a) Principle:

The samples are heatedin neutral detergent solution that dissolves the cell contents and the residue left is known as NDF.

b) Reagents:

Preparation of 1 L neutral detergent solution (NDS)

Ethylene diamino tetra acetate disodium dihydrate (EDTA-Na_2)	18.15 g
Sodium lauryl sulphate	30 g
Dibasic sodium phosphate	4.55 g
Sodium borate ($Na_2B_4O_7.10H_2O$)	6.80 g
Ethylene glycol mono ethyl ether (2-ethoxyethanol)	10 ml

Neutral detergent solution (NDS)

18.15 g of EDTA-Na_2 and 6.80 g of $Na_2B_4O_7.10H_2O$ were heated in about 700 ml distilled water until completely dissolved. After that, added 30 g of sodium lauryl sulphate

and 10 ml of 2-ethoxyethanol to this solution. Dissolve 4.56 g dibasic sodium phosphate in 100 ml of dw separately in another beaker. The two solutions were mixed thoroughly, made upto 1 L using distilled water and adjusted the pH (6.9-7.1).

c) Estimation:

1.0 g of sample was taken in glass crucibles and fixed to fibre estimation system (Fibra Plus). Then 100 ml of NDS was added, the initial temperature of the instrument was fixed at 500°C and then reduced to 250-300°C when the boiling of the samples had just started. The samples were heated for 1 hr, the remaining solution was drained and the samples were washed thoroughly with distilled water. The crucibles were then oven dried and cooled in a desicator and weighed. Neutral detergent fibre was expressed in percent and was calculated from the following formula:

$$\text{Neutral detergent fibre} = \frac{(\text{Weight of crucible} + \text{fibre}) - (\text{Weight of crucible})}{\text{Weight of sample (g)}} \times 100$$

3.7.9 *In vitro* dry matter digestibility (IVDMD) (Tilley and Terry 1963)

a) Reagents:

i) Preparation of Solution A and B

Solution A:

Sodium hydrogen carbonate	49.0g
Disodium hydrogen orthophosphate (anhydrous)	18.5g

The two reagents were dissolved in distilled water to make final vol.upto 1 L.

Solution B

Potassium chloride	28.5g
Sodium chloride	23.5g
Calcium chloride	2g
Magnesium chloride	6g

The above reagents were dissolved in distilled water to make final volume upto 1 L.

ii) **Stock solution:** Solution B was added to Solution A in the ratio 9: 1 to make a total vol. of 1 L. Solution B was added only after the bicarbonate and phosphate in solution A has been completely dissolved.

iii) **Solution C:** It was prepared immediately before use by diluting 1 part of the above stock solution with 4 parts of dw and warmed up 39±1°C. CO_2 was bubbled in the cloudy solution until the solution became clear.

iv) **Buffer nutrient solution:** It was prepared immediately before use by mixing strained rumen liquor and solution C in the ratio 1:4.

b) Estimation:

0.5g of oven dried sample and about 50 ml of nutrient buffer solution were taken in a centrifuge tube (250 ml) having a gas release valve. The carbon dioxide gas was passed for

about 10 seconds over the surface of the tube contents.The tube was immediately stoppered and incubated for 48 hours at 39°C. During incubation, the tubes were gently swirled to re-suspend the substrate. The tubes were filtered after incubation and the residue was then treated with 50 ml of pepsin (0.2%) in hydrochloric acid (0.1 N). The tubes were again incubated at 39°C for 24 hrs. The tubes were then filtered through pre-weighed crucibles. The residue was dried for overnight at 100°C and weight was recorded. *In vitro* dry matter digestibility was expressed in percent and determined from the formula given below:

$$\textit{In vitro}\text{ dry matter digestibility} = \frac{w_3 - w_4}{w_3} \times 100$$

where: w_1 = wt. of empty crucible

w_2 = wt. of crucible + residual material

w_3 = wt. of sample taken

$w_2 - w_1 = w_4$ = wt. of residual matter

3.8 Antinutritional composition of oat samples

3.8.1 Nitrate-N (Cotaldo *et al* 1975)

a) Principle:

The complex is formed under highly acidic conditions by nitration of salicylic acid which absorbs maximum at 410 nm in basic (pH above 12) solution. The amount of nitrate-N present is directly proportional to the absorbance of chromophore. Nitrite, chloride and ammonium ions do not interfere.

b) Reagents:

i) Sodium hydroxide (2N)

ii) Salicylic acid (5%) -H_2SO_4

iii) KNO_3 standard (0-50 µg)

c) Extraction and Estimation:

0.1 g of dried sample was extracted in 10 ml of dw and the tubes were incubated at 60-70 °C for 1 hr. The samples were filtered and the filtrate (0.2ml) was taken in the tubes for the estimation. To this, added 0.8 ml of Salicylic acid (5%) -H_2SO_4 (w/v). The contents were thoroughly mixed and placed for 20 minutes at room temp. About 19 ml of sodium hydroxide (2N) was added to raise the pH (>12) and then samples were cooled at room temperature. The OD was taken at 410 nm and the quantity of nitrate-N was estimated using 0-50 µg of KNO_3 as standard.

3.8.2 Nitrite-N (Guevara *et al* 1988)

a) Reagents:

i) **1% (w/v) Sulfanilamide ($C_6H_8N_2O_2S$) in 1N HCl:** Dissolve 1 g Sulfanilamide in 100 ml of 1 N hydrochloric acid.

ii) 0.02% N-(1-napthyl) Ethylene Diamine Dihydrochloride (NEDD)

b) Extraction and Estimation:

0.1g of dried sample was extracted in 10 ml of dw the tubes were incubated at 60-70°C for 1 hr. The tubes were filtered and diluted 1 ml sample was taken for estimation. To this added, 1 ml of $C_6H_8N_2O_2S$ (1%) and 1 ml of NEDD (0.02%). The tubes were incubated at 30°C for 30 minutes and thereafter vol. was made 5.0 ml with dw. The OD was taken at 540 nm and the quantity of nitrite-N was estimated prepared using $NaNO_2$ (0-10 µg) as standard.

3.9 Yield and yield parameters

The observations were recorded at 60 DAS.

3.9.1 Leaf Length

Five leaves were selected randomly from each treatment and length was recorded in centimetres.

3.9.2 Leaf Breadth

Five leaves were selected randomly from each treatment and breadth was recorded in centimetres.

3.9.3 Plant Height

Five leaves were selected randomly from each treatment and height was recorded in centimetres.

3.9.4 Tillers sq m^{-1}

Tillers of randomly selected row from each treatment were counted.

3.9.5 Number of leaves per plant

Five plants were selected randomly from each treatment and number of leaves were counted.

3.9.6 Green Fodder yield (GFY)

The crop was harvested and GFY was recorded for each plot. It was expressed in q/ha.

3.9.7 Dry matter yield (DMY)

DMY of each treatment was analysed from DM content and green fodder yield. It was expressed in q/ha.

3.9.8 Crude protein yield (CPY)

CPY of each treatment was analysed from DMY and crude protein content. It was expressed in q/ha.

3.10 Statistical analysis

The data was statistically analysed by ANOVA. The mean separation of treatments were done by using Tukey's post hoc test. All data analysis was carried out through SAS software Version 9.3.

CHAPTER IV

RESULTS AND DISCUSSION

The present investigation evaluated the effect of six planting dates and three growth stages in oat genotypes. The results obtained have been discussed under the following sub-headings:

4.1 Effect of different planting dates and growth stages on nitrogen metabolism enzymes in oat genotypes.

4.2 Effect of different planting dates and growth stages on nutritional composition, digestibility and anitnutritional composition in oat genotypes.

4.3 Effect of different planting dates on yield and yield parameters in oat genotypes.

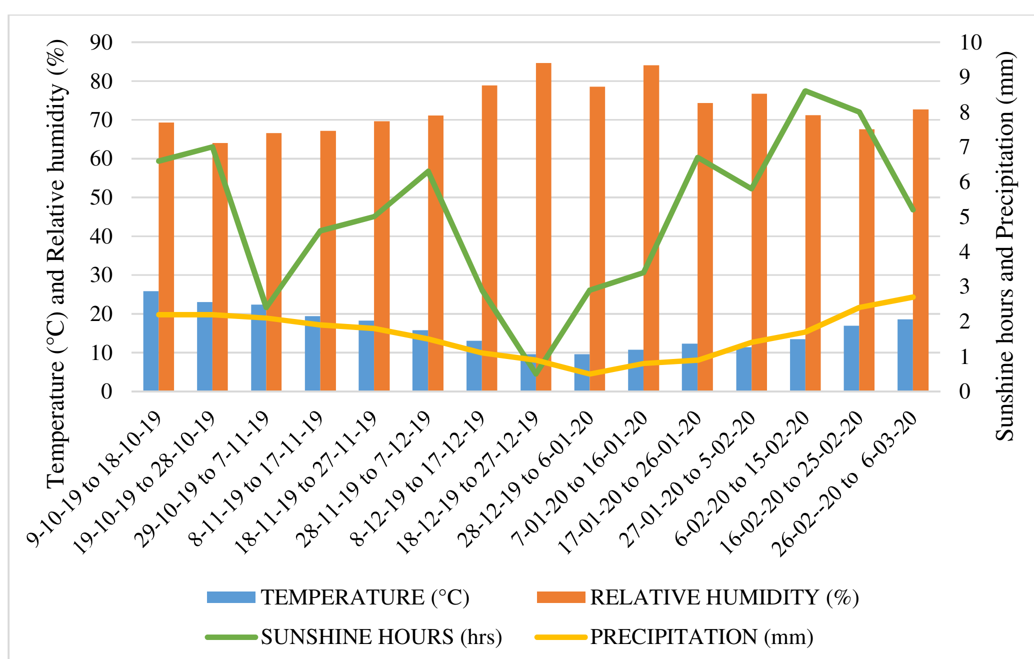


Fig 1 Meterological data during the experimental period

4.1 Effect of different planting dates and growth stages on nitrogen metabolism enzymes in oat genotypes.

4.1.1 Nitrate Reductase (NR)

NR is a substrate inducible enzyme and serve as a marker enzyme to judge the ability of plant to utilize soil nitrogen (Raimanova and Trikova 2007). NR consists of five conserved domains i.e. Mo-MPT domain, a dimer interface domain, a cytochrome b domain, NADH domain that combines with FAD domain (Campbell 1999). In our study, the NR activity depicted significant ($F = 507.5$, $P<0.01$) relationship with staggered planting dates. Highest NR activity was exhibited at Oct 9 planting date (18.90 µmol hr^{-1} g^{-1} FW) whereas lowest NR activity was exhibited at Nov 26 planting date (6.20 µmol hr^{-1} g^{-1} FW) (Table 1). A recent study also reported influence of seasonal variations on NR activity and observed higher NR

activity in warmer months (Prakash and Saxena 2020). As the green fodder yield was 46-58% and 45.3-52.5% reduced in the later planting dates, the reduced growth, low temperature and less sunshine hours may be responsible for lower NR activity during late planting dates as compared to optimum (Oct 24) and sub-optimum planting dates (Oct 9 and Nov 13). Adverse weather conditions such as of low temperature leads to lesser stability of the enzyme because electron transport among redox centres and FAD domain are sensitive to low temperature (Gao *et al* 2000). Another study reported that low temperature stress prevailing during the growth of the plant resulted in disturbed electron transfer throughout the NR channel at the interdomain site between heme and MoCo domains (Aydin and Nalbantoglu 2010). NR activity is also highly dependent upon light because of its sensitivity towards dark conditions (Bian *et al* 2020). Light enhanced the expression of NR related gene, making available the reductants (ATP and NADPH) needed for nitrate reduction (Iglesias-Bartolome *et al* 2004). In plants, 25% of the ATP and NADPH produced during photosynthetic electron transport are used for nitrate assimilation (Walker *et al* 2014). In our study also, the photosynthetic machinery may be slowed down and likewise the NR activity in late planting experiencing low temperature and sunshine hours. The efficient assimilation of nitrate by upregulated NR activity during initial planting dates (Oct 9, Oct 24 and Nov 13) may be responsible for lower nitrate accumulation and the opposite trend was observed during late planting dates where nitrate accumulation was high due to lower NR activity.

NR activity also depicted significant ($F = 22.1$, $P<0.01$) relationship with growth stages and lied in the range from 9.09-13.21 µmol hr^{-1} g^{-1} FW. The present study observed that the enzyme activity varied according to the sunshine hours during the growth stages butshowed no specific trend with the maturity of the plant. The present study found positive correlation with sunshine hours ($r=0.551$, $P<0.05$) at Oct 9 planting date and also with minimum temperature at the growth stages of Oct 9 ($r= 0.763$, $P<0.01$) and Nov 13 ($r=0.836$, $P<0.05$) planting dates. Several workers reported influence of seasons and environmental conditions on the nitrate reductase enzyme activity (Larmure and Munier-Jolain 2019, Hofmann *et al* 2013, Young *et al* 2007, Zhao *et al* 2006).

NR activity was significantly ($F = 752.0$, $P<0.01$) high in OL-10 genotype at almost all the planting dates and growth stages in comparison with OL-11 genotype. OL-10 being a fast growing genotype requires more nutrients so it may uptake and assimilate more nitrate as compared to slow growing genotype (OL-11). Genotypic differences for NR activity was also reported by earlier workers (Kaur *et al* 2015, Kaur and Goyal 2016 and Shah *et al* 2017). The three-way interaction of planting dates, growth stages and genotypes was found to be significant ($F = 14.9$, $P<0.01$).

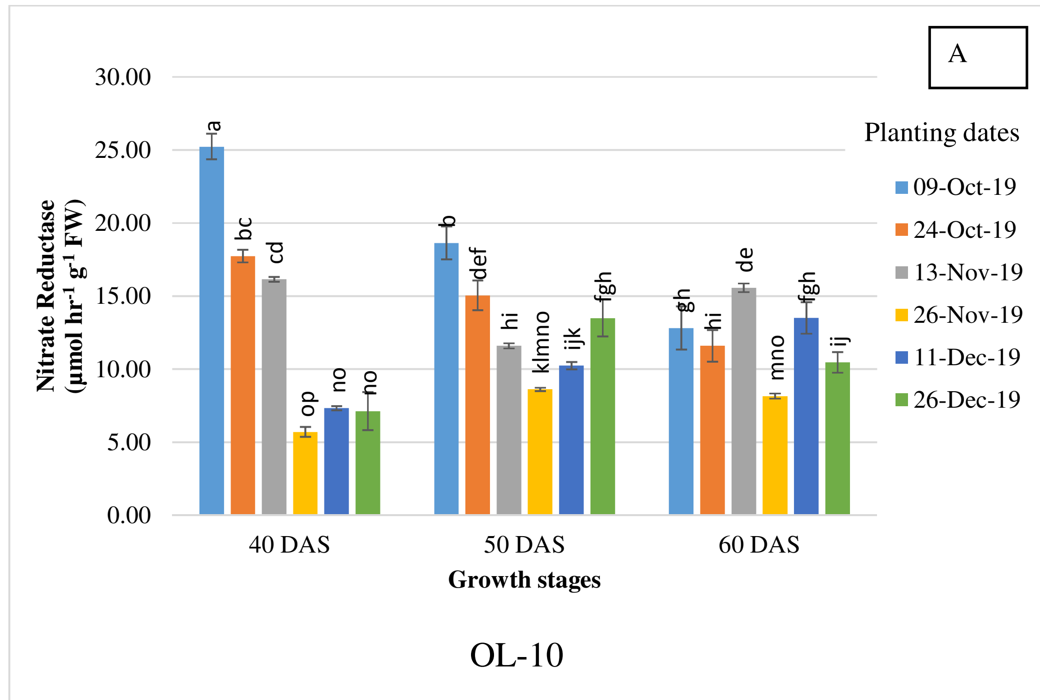
Table 1 Nitrate reductase activity (µmol hr^{-1} g^{-1} FW) as influenced by planting dates and growth stages in oat genotypes.

Nitrate reductase (µmol hr^{-1} g^{-1} FW)					
Genotype	**Planting dates**	**Growth stages (DAS)**			**Mean**
		40	**50**	**60**	
OL-10	09-Oct-19	25.23±0.86^{a}	18.64±1.13^{b}	12.83±1.49^{gh}	**18.90^{a}**
	24-Oct-19	17.74±0.44^{bc}	15.05±1.02^{def}	11.60±0.29^{hi}	**14.80^{b}**
	13-Nov-19	16.15±0.16^{cd}	11.60±0.18^{hi}	15.57±0.30^{de}	**14.44^{b}**
	26-Nov-19	5.71±0.34^{op}	8.62±0.11^{klmno}	8.16±0.17^{mno}	**7.49^{g}**
	11-Dec-19	7.33±0.14^{no}	10.25±0.26^{ijk}	13.52±1.08^{fgh}	**10.37^{e}**
	26-Dec-19	7.13±0.81^{no}	13.51±1.27^{fgh}	10.47±0.16^{ij}	**10.37^{e}**
Mean		**13.21^{a}**	**12.94^{a}**	**12.03^{b}**	
OL-11	09-Oct-19	15.80±0.62^{cde}	13.36±1.08^{ghi}	10.07±0.78^{ijkl}	**13.08^{c}**
	24-Oct-19	12.53±0.50^{gh}	8.61±0.40^{klmno}	7.39±0.58^{no}	**9.50^{ef}**
	13-Nov-19	13.95±0.33^{efg}	8.25±0.14^{lmno}	13.05±0.12^{fgh}	**11.75^{d}**
	26-Nov-19	4.70±0.14^{p}	8.09±0.06^{mno}	5.83±0.29^{op}	**6.20^{h}**
	11-Dec-19	4.18±0.10^{p}	8.57±0.22^{klmno}	9.58±0.76^{jklmn}	**7.44^{g}**
	26-Dec-19	7.72±0.20^{nop}	9.87±0.38^{ijkl}	8.65±0.38^{klmno}	**8.74^{f}**
Mean		**9.81^{c}**	**9.46^{cd}**	**9.09^{d}**	
CD at 5%	Planting dates (PD) = 0.574, Growth stages (GS) = 0.332, Genotypes (G) = 0.226, PD x GS = 0.989, PD x G = 0.857, GS x G = NS, PD x GS x G = 1.104				

Values are mean ± standard deviation of three replications

NS = Non-significant

Values with same letter(s) are non-significant at 5% level of significance (Tukey's post-hoc test)



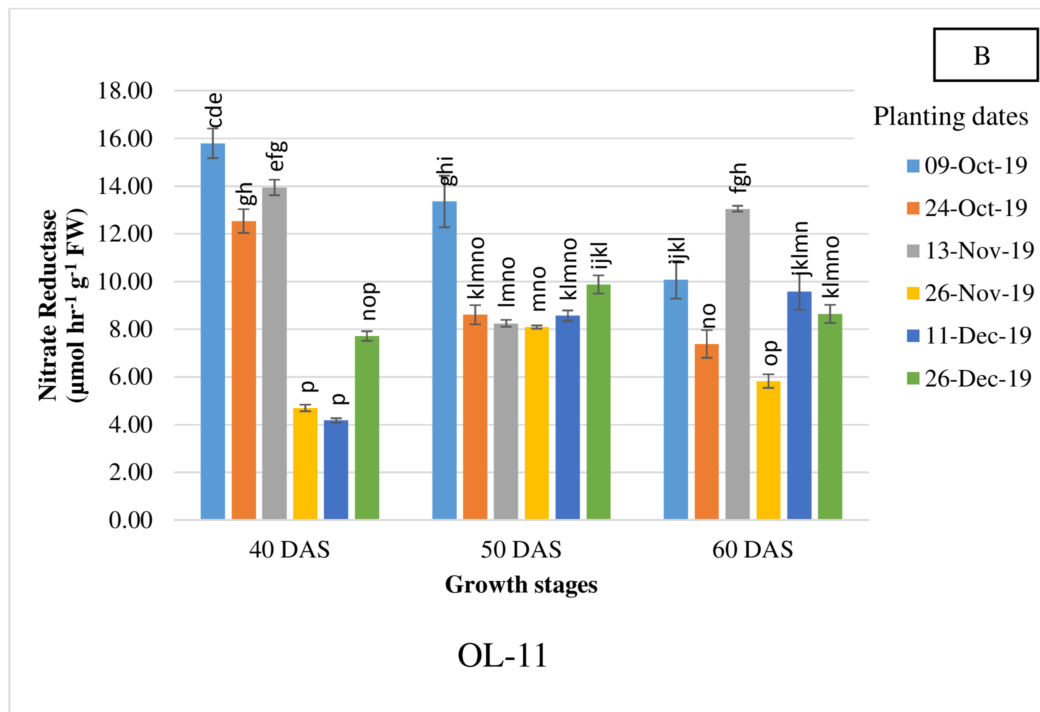


Fig 2 Nitrate reductase activity (µmol hr^{-1} g^{-1} FW) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes (Error bars represents standard error)

4.1.2 Nitrite Reductase

Nitrite reductase moves in coordination with nitrate reductase since the product of nitrate reductase is used as substrate for nitrite reductase and the gene expression of both enzymes are also regulated in similar manner (Pathak *et al* 2011). The assimilation of nitrite should be much higher than nitrate so that all the nitrite formed by NR is completely reduced to ammonia since nitrite is considered toxic to plant cell (Liao *et al* 2019). The present study observed similar trend for nitrite reductase (NiR) as that for nitrate reductase (NR). Significant ($F = 456.6$, $P<0.01$) variation was observed for nitrite reductase activity with staggered planting dates. Among six planting dates, nitrite reductase activity lied in the range from 88.3-250.1 µmol hr^{-1} g^{-1} FW (Table 2). Highest NiR activity was exhibited at Oct 9 planting date and lowest NiR activity was exhibited at Nov 26 planting date. Previous study

Table 2 Nitrite reductase activity (µmol hr^{-1} g^{-1} FW) as influenced by planting dates and growth stages in oat genotypes.

Nitrite reductase (µmol hr^{-1} g^{-1} FW)					
Genotype	**Planting dates**	**Growth stages (DAS)**			**Mean**
		40	**50**	**60**	
OL-10	09-Oct-19	302.9±3.44^{a}	267.1±18.55^{bc}	180.4±2.19^{ghi}	**250.1^{a}**
	24-Oct-19	283.8±1.65^{ab}	162.9±5.24^{hij}	84.6±2.82^{uvw}	**177.1^{c}**
	13-Nov-19	230.2±7.71^{de}	144.6±10.5^{jklm}	149.2±8.62^{jkl}	**174.7^{c}**
	26-Nov-19	93.1±6.53^{stuvw}	156.7±4.85^{ijk}	147.9±0.95^{jkl}	**132.6^{fg}**
	11-Dec-19	136.0±1.57^{klmno}	137.3±2.19^{klmn}	195.8±5.20^{fg}	**156.4^{d}**
	26-Dec-19	103.3±0.36^{qrstuv}	235.2±12.14^{d}	175.0±7.21^{ghi}	**171.2^{c}**
Mean		**191.6^{a}**	**184.0^{b}**	**155.5^{d}**	
OL-11	09-Oct-19	250.4±6.88^{cd}	185.6±11.47^{fgh}	140.6±1.88^{jklmn}	**192.2^{b}**
	24-Oct-19	243.3±5.67^{cd}	106.5±6.29^{qrstu}	74.0±1.57^{w}	**141.3^{ef}**
	13-Nov-19	208.1±8.75^{ef}	113.1±10.84^{opqrs}	123.1±5.12^{mnopq}	**148.1^{de}**
	26-Nov-19	80.0±1.25^{vw}	96.7±4.43^{rstuvw}	88.3±2.37^{tuvw}	**88.3^{i}**
	11-Dec-19	101.7±9.49^{qrstuv}	117.5±2.25^{nopqr}	130.6±3.90^{lmnop}	**116.6^{h}**
	26-Dec-19	109.2±7.56^{pqrst}	142.7±15.26^{jklm}	133.5±6.68^{klmno}	**128.5^{g}**
Mean		**165.5^{c}**	**127.0^{c}**	**115.0^{f}**	
CD at 5%	Planting dates (PD) = 7.14, Growth stages (GS) = 4.13, Genotypes (G) = 2.81, PD x GS = 12.49, PD x G = 11.65, GS x G = 7.14, PD x GS x G = 13.01				

Values are mean ± standard deviation of three replications

NS = Non-significant

Values with same letter(s) are non-significant at 5% level of significance (Tukey's post-hoc test)

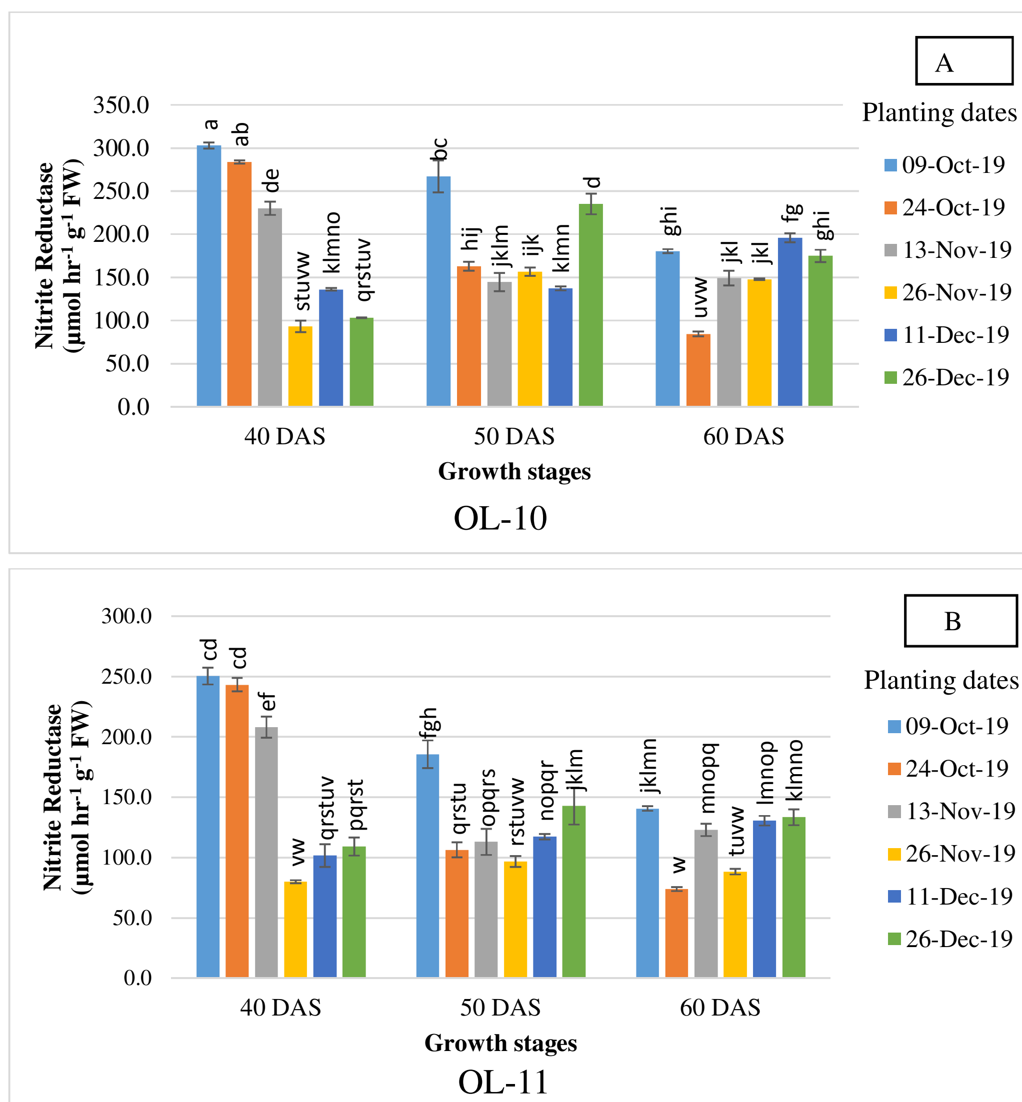


Fig 3 Nitrite reductase activity (µmol hr^{-1} g^{-1} FW) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes (Error bars represents standard error)

reported that conversion of nitrite to ammonium is very less under low intensity of light (Kaiser *et al* 2011). The NiR enzyme activity predominantly depends on ferredoxin for electrons which is supplied through light by photosystem I as a product of photosynthetic electron transport (Ali 2020). In our study also, during low intensity of sunlight, the photosynthetic machinery may be slowed down and likewise the NiR activity. The nitrite is efficiently assimilated by upregulated nitrite reductase activity during initial planting dates (Oct 9, Oct 24 and Nov 13) which may be responsible for low nitrite content but the opposite trend was observed during late planting dates where nitrite content was high because of low activity of NiR. This may be due to less sunshine hours and low temperature at later planting dates. A significant association was observed between sunshine hours, temperature and NiR.

The NiR depicted positive correlation with NR at almost all the planting dates (Oct 9, Nov 26 and Dec 11).

Significant (F = 315.3, P<0.01) variation was observed for NiR activity with growth stages. Among the growth stages, NiR activity lied in the range between 115.0-191.6 µmol hr^1 g^{-1} FW. Similar to NR, NiR activity varied according to the sunshine hours during the growth stages but showed no specific trend with the maturity of the plant. NiR activity was positively correlated with sunshine hours at Oct 9 planting date (r=0.807, P<0.01) and Oct 24 plating date (r=0.967, P<0.01) and also with minimum temperature at Oct 9 planting date (r=0.971, P<0.01).

NiR activity was significantly (F = 856.0, P<0.01) higher in OL-10 genotype at almost all the planting dates and growth stages as compared to OL-11 genotype because of higher ability to assimilate nitrite as it is a fast growing genotype. Genotype variations for NiR activity was also observed by Shah *et al* (2017) in barley. The three way interaction of planting dates, growth stages and genotypes was found to be significant (F = 18.4, P<0.01).

4.1.3 Glutamine Synthetase

Glutamine synthetase is considered as a multi-functional enzyme as it plays a vital role in ammonia assimilation and in the formation of proteins (Maxclaus-Daubresse *et al* 2006). The ammonium ions are incorporated by GS enzyme from variety of sources such as nitrite reduction, photorespiration, degradation of nitrogenous compounds, metabolism of phenylpropanoids and direct absorption from the soil (Hakeem *et al* 2012). The ammonia assimilated by GS is supplied in constant amount to GOGAT and this enzyme was also considered as rate limiting enzyme of ammonia assimilation (Mokhele *et al* 2012). The present study reported significant (F = 665.9, P<0.01) variation in GS activity with staggered planting dates. Among six planting dates, GS activity lied in the range from 1.20-1.79 mmol min^{-1} g^{-1} FW (Table 3). Highest GS activity was reported at Dec 11 planting date and lowest at Oct 9 planting date. Ali (2020) suggested that variability in growing season and environmental conditions affects the GS activity. GS enzyme exists in two isoforms i.e. GS1 and GS2 and GS1 isoform is mainly responsible for reassimilation of ammonia when photosynthesis got declined and GS2 assimilates the ammonia formed during photorespiration (Habash *et al* 2001). In our study, as low temperature conditions prevailed during late planting, GS1 may be responsible for increased GS activity at these planting dates. This in turn may be due to utilization of existing carbon–skeleton in N metabolism (Liu *et al* 2017). Further demand of proline, an osmolyte was more in adverse environmental conditions and glutamate is the substrate for its synthesis (Lu *et al* 2005 and Yan *et al* 2006). Similarly, in our study, the GS activity may be increased with low temperature stress to provide the constant glutamate pool for proline synthesis via the formation of glutamine. Larher *et al* (1998) also found some association between proline synthesis and GS activity. In the present

study, GS activity showed inverse relationship with temperature.

The GS activity also varied significantly (F = 7.9, P<0.01) with growth stages and lied in the range from 1.45-1.56 mmol min^{-1} g^{-1} FW. No clear trend was observed during initial planting dates but during the late planting dates (Nov 26, Dec 11 and Dec 26), GS activity increased with the maturity of the plant. Previous studies also reported increased GS activity with leaf ageing (Kaur and Goyal 2016 and Ghisi *et al* 1999).

The OL-10 exhibited significantly (F = 556.9, P<0.01) higher GS activity at almost all the planting dates and growth stages as it can assimilate more ammonium because it is a fast growing genotype. Genotypic differences were also observed by several other workers (Jallouli *et al* 2019, Fortunato *et al* 2019 and Shah *et al* 2017). The three way interaction of planting dates, growth stages and genotypes was found to be significant (F = 15.2, P<0.01).

Table 3 Glutamine synthetase activity (mmol min^{-1} g^{-1} FW) as influenced by planting dates and growth stages in oat genotypes.

		Glutamine synthetase (mmol min^{-1} g^{-1} FW)			
Genotype	**Planting dates**	**Growth stages (DAS)**			**Mean**
		40	**50**	**60**	
OL-10	09-Oct-19	1.73 ± 0.03^{e}	1.05 ± 0.05^{s}	1.30 ± 0.02^{pq}	$\mathbf{1.36^{ef}}$
	24-Oct-19	1.69 ± 0.02^{ef}	1.87 ± 0.04^{cd}	1.01 ± 0.02^{s}	$\mathbf{1.52^{d}}$
	13-Nov-19	1.87 ± 0.02^{cd}	1.49 ± 0.03^{kl}	1.22 ± 0.01^{qr}	$\mathbf{1.53^{d}}$
	26-Nov-19	1.48 ± 0.01^{klm}	1.83 ± 0.02^{d}	1.89 ± 0.02^{bcd}	$\mathbf{1.73^{b}}$
	11-Dec-19	1.38 ± 0.03^{no}	1.96 ± 0.04^{ab}	2.01 ± 0.03^{a}	$\mathbf{1.79^{a}}$
	26-Dec-19	1.43 ± 0.02^{lmno}	1.58 ± 0.03^{ghi}	1.92 ± 0.02^{bc}	$\mathbf{1.64^{c}}$
Mean		$\mathbf{1.60^{b}}$	$\mathbf{1.63^{a}}$	$\mathbf{1.56^{c}}$	
OL-11	09-Oct-19	1.50 ± 0.02^{jkl}	0.92 ± 0.02^{t}	1.17 ± 0.01^{r}	$\mathbf{1.20^{g}}$
	24-Oct-19	1.49 ± 0.01^{jkl}	1.57 ± 0.01^{hij}	0.93 ± 0.02^{t}	$\mathbf{1.33^{f}}$
	13-Nov-19	1.62 ± 0.01^{fgh}	1.37 ± 0.02^{op}	1.17 ± 0.00^{r}	$\mathbf{1.39^{e}}$
	26-Nov-19	1.40 ± 0.02^{mno}	1.53 ± 0.02^{ijk}	1.65 ± 0.04^{efg}	$\mathbf{1.53^{d}}$
	11-Dec-19	1.24 ± 0.04^{qr}	1.89 ± 0.01^{bcd}	2.02 ± 0.02^{a}	$\mathbf{1.72^{b}}$
	26-Dec-19	1.43 ± 0.01^{lmno}	1.46 ± 0.04^{klmn}	1.96 ± 0.01^{ab}	$\mathbf{1.62^{c}}$
Mean		$\mathbf{1.45^{e}}$	$\mathbf{1.46^{de}}$	$\mathbf{1.48^{d}}$	
CD at 5%	Planting dates (PD) = 0.023, Growth stages (GS) = 0.014, Genotypes (G) =0.009 , PD x GS = 0.041, PD x G = 0.038, GS x G = 0.023, PD x GS x G = 0.043				

Values are mean ± standard deviation of three replications

NS = Non-significant

Values with same letter(s) are non-significant at 5% level of significance (Tukey's post-hoc test)

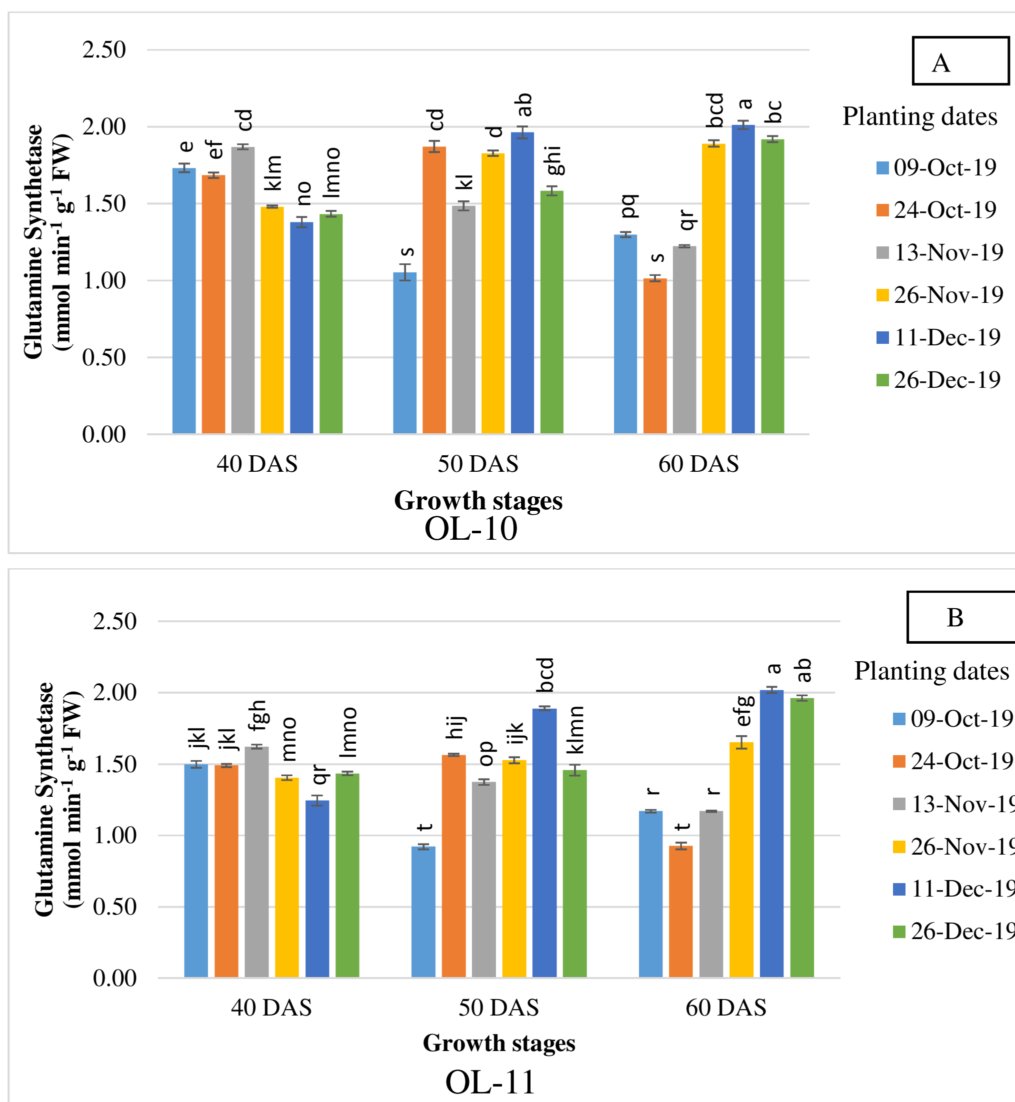


Fig 4 Glutamine synthetase activity (mmol min^{-1} g^{-1} FW) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes (Error bars represents standard error)

4.1.4 Glutamate synthase (GOGAT)

Glutamate synthase works in parallel with GS and together constitutes GS/GOGAT cycle. The uptake of ammonia by the plants with the help of ammonia transporters is energetically less expensive as compared to nitrate reduction to ammonia (Goel and Singh 2015). Ammonia is toxic to plant cell so needs to be instantly metabolized even at low concentrations (Wang *et al* 2007). The combination of these two enzymes leads to rapid assimilation of ammonia into numerous N-containing compounds such as amino acids, proteins and nucleotides (Nagy *et al* 2013). In our study, GOGAT activity significantly (F = 2195.9, P<0.01) varied among staggered planting dates and lied in the range between 0.676-1.794 mmol min^{-1} g^{-1} FW (Table 4). Highest GOGAT activity was observed at Nov 26

planting date and lowest activity was observed at Dec 26 planting date. No decline in GOGAT activity was observed with low temperature similar to that of GS. The GOGAT activity was positively correlated with GS at almost all the planting dates.

GOGAT activity also varied significantly (F = 124.7, P<0.01) with growth stages. No clear trend of GOGAT activity was observed with growth stages during the first three planting dates but it was observed that the activity got increased with the maturity of the plant during later planting dates. Kaur and Goyal (2016) also reported similar trend in GOGAT activity from 30 to 60 DAS. GOGAT activity was significantly (F = 254.2, P<0.01) higher in OL-10 genotype at almost all the planting dates and growth stages because of higher ability to assimilate ammonia, being a fast growing genotype. Significant differences among genotypes were observed by previous workers (Shah *et al* 2017, Imran *et al* 2019). The three way interaction of planting dates, growth stages and genotypes was found to be significant (F=20.1, P<0.01).

Table 4 Glutamate synthase activity (mmol min^{-1} g^{-1} FW) as influenced by planting dates and growth stages in oat genotypes

Glutamate synthase (mmol min^{-1} g^{-1} FW)					
Genotype	**Planting dates**	**Growth stages (DAS)**			
		40	**50**	**60**	**Mean**
OL-10	09-Oct-19	1.100±0.08^{d}	0.753±0.25^{ijkl}	0.980±0.06^{def}	**0.944^{d}**
	24-Oct-19	0.683±0.05^{klmn}	1.040±0.07^{de}	0.583±0.01^{no}	**0.769^{f}**
	13-Nov-19	1.047±0.02^{de}	0.953±0.02^{efg}	0.593±0.02^{no}	**0.864^{e}**
	26-Nov-19	1.620±0.04^{b}	1.820±0.02^{a}	1.876±0.01^{a}	**1.772^{a}**
	11-Dec-19	0.853±0.02^{ghi}	1.100±0.04^{d}	1.457±0.04^{c}	**1.137^{b}**
	26-Dec-19	0.586±0.02^{no}	0.807±0.05^{hij}	0.897±0.05^{fgh}	**0.763^{f}**
Mean		**0.982^{b}**	**1.079^{a}**	**1.064^{a}**	
OL-11	09-Oct-19	0.658±0.06^{lmn}	0.860±0.02^{fghi}	0.803±0.02^{hijk}	**0.773^{f}**
	24-Oct-19	0.607±0.07^{mno}	0.817±0.02^{hij}	0.447±0.02^{p}	**0.623^{g}**
	11-Nov-19	0.807±0.03^{hij}	0.683±0.05^{klmn}	0.530±0.02^{op}	**0.673^{g}**
	26-Nov-19	1.656±0.05^{b}	1.913±0.03^{a}	1.813±0.02^{a}	**1.794^{a}**
	11-Dec-19	0.727±0.03^{jklm}	0.947±0.01^{efg}	1.380±0.05^{c}	**1.018^{c}**
	26-Dec-19	0.500±0.02^{op}	0.683±0.03^{klmn}	0.843±0.04^{ghij}	**0.676^{g}**
Mean		**0.826^{c}**	**0.984^{b}**	**0.969^{b}**	
CD at 5%	Planting dates (PD) = 0.037, Growth stages (GS) = 0.021 , Genotypes (G) = 0.014, PD x GS = 0.064, PD x G = 0.060, GS x G = 0.037 , PD x GS x G = 0.067				

Values are mean ± standard deviation of three replications
NS = Non-significant
Values with same letter(s) are non-significant at 5% level of significance (Tukey's post-hoc test)

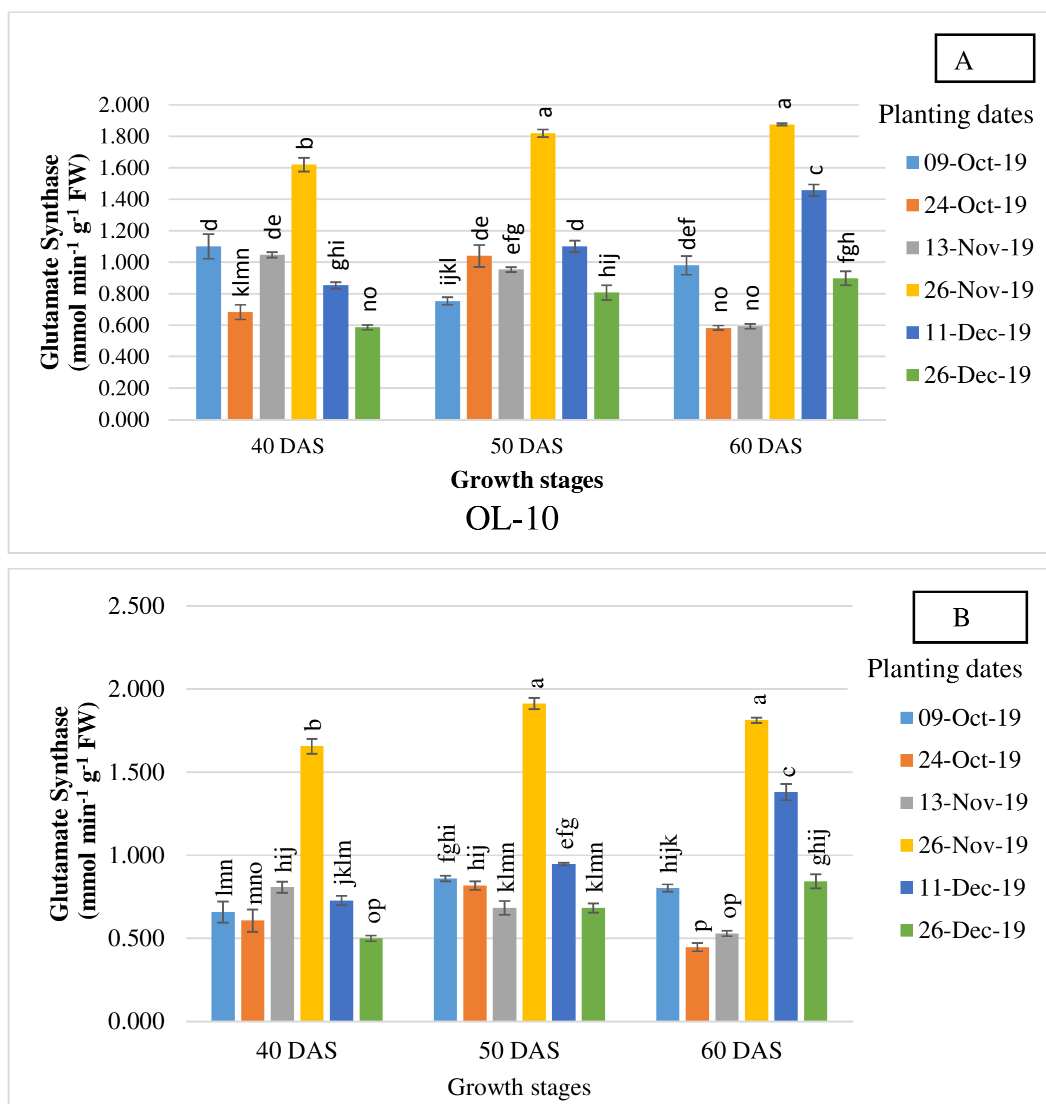


Fig 5 **Glutamate synthase activity (mmol min^{-1} g^{-1} FW) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes** (Error bars represents standard error)

4.1.5 Glutamate Dehydrogenase (GDH)

Glutamate dehydrogenase is an enzyme which catalyses the reversible reaction i.e. anabolic reaction, the formation of glutamate and catabolic reaction, the formation of ammonia and α-ketoglutarate. GDH is primarily known for maintaining the balance between carbon and nitrogen pools (Miflin and Habash 2002). In our study, GDH activity varied significantly ($F = 161.4$, $P<0.01$) with staggered planting dates. Among six planting dates, GDH activity lied in the range from 0.817-1.461 mmol min^{-1} g^{-1} FW (Table 5). Highest GDH activity was observed at Nov 26 planting date in OL-10 genotype and at Dec 11 planting date in OL-11 genotype. During adverse environmental conditions such as of low temperature, GDH is considered as an alternative enzyme to GS/GOGAT cycle (El-Shora and Ebo-Kareem 2001). The generation of C-skeleton (α-ketoglutarate) is maintained during low temperature

stress via the constant activity of isocitrate dehydrogenase, a TCA cycle enzyme (Liu *et al* 2017). Likewise, in our study this available carbon skeleton may be utilized in the ammonium assimilation during late planting dates via the GDH activity. Lu *et al* (2005) reported decline in the GDH activity with low temperature stress in rice. In contrast, our results presented uplifted GDH activity with low temperature stress, depicting a different response of ammonia assimilation process in comparison with rice. Another study reported that the enhanced expression of the genes involved in cold resistance were responsible for upregulated GDH activity (Goel and Singh 2015). The present study observed inverse relationship of GDH activity with minimum temperature at Oct 9 planting date and positive correlation with GS and GOGAT at almost all the planting dates.

GDH activity also depicted significant (F = 416.3, P<0.01) relationship among growth stages. No clear trend was observed during initial four planting dates but the GDH activity increased with maturity during the later planting dates (Dec 11 and Dec 26). GDH activity was significantly (F=416.3, P<0.01) high in the fast growing genotype (OL-10) in comparison with slow growing genotype (OL-11). Previous workers also reported significant difference in GDH activity among genotypes (Wang *et al* 2016, Guellim *et al* 2019 and Singla *et al* 2020). The three way interaction between planting dates, growth stages and genotypes was found to be significant (F=19.5, P<0.01).

Table 5 Glutamate dehydrogenase activity (mmol min^{-1} g^{-1} FW) as influenced by planting dates and growth stages in oat genotypes.

Glutamate dehydrogenase (mmol min^{-1} g^{-1} FW)					
Genotype	**Planting dates**	**Growth stages (DAS)**			
		40	**50**	**60**	**Mean**
OL-10	09-Oct-19	0.855±0.05^{ijkl}	1.045±0.06^{defg}	1.021±0.06^{efgh}	**0.974^{d}**
	24-Oct-19	0.765±0.07^{jklm}	1.176±0.02^{cde}	0.588±0.02^{nopq}	**0.843^{ef}**
	13-Nov-19	1.061±0.07^{cdefg}	1.011±0.02^{fghi}	0.634±0.02^{mnop}	**0.902^{de}**
	26-Nov-19	1.994±0.08^{a}	1.192±0.09^{cd}	1.198±0.03^{cd}	**1.461^{a}**
	11-Dec-19	0.743±0.04^{klmn}	1.208±0.11^{c}	1.478±0.07^{b}	**1.143^{b}**
	26-Dec-19	0.700±0.03^{lmnop}	0.837±0.01^{jkl}	0.914±0.01^{ghij}	**0.900^{de}**
Mean		**1.020^{b}**	**1.078^{a}**	**0.972^{b}**	
OL-11	09-Oct-19	0.741±0.04^{klmn}	0.914±0.04^{ghij}	0.877±0.04^{hijk}	**0.844^{ef}**
	24-Oct-19	0.815±0.03^{jkl}	0.762±0.01^{jklm}	0.433±0.04^{q}	**0.670^{h}**
	13-Nov-19	0.813±0.03^{jkl}	0.850±0.08^{ijkl}	0.575±0.01^{opq}	**0.745^{gh}**
	26-Nov-19	0.914±0.07^{ghij}	0.540±0.06^{pq}	0.815±0.03^{jkl}	**0.756^{g}**
	11-Dec-19	0.610±0.01^{mnop}	1.152±0.04^{cdef}	1.428±0.02^{b}	**1.063^{c}**
	26-Dec-19	0.735±0.06^{klmno}	0.880±0.02^{hijk}	1.085±0.03^{cdef}	**0.817^{fg}**
Mean		**0.771^{d}**	**0.849^{c}**	**0.869^{c}**	
CD at 5%	Planting dates (PD) = 0.048, Growth stages (GS) = 0.028, Genotypes (G) = 0.019, PD x GS = 0.084, PD x G = 0.079, GS x G = 0.048 , PD x GS x G = 0.088				

Values are mean ± standard deviation of three replications
NS = Non-significant
Values with same letter(s) are non-significant at 5% level of significance (Tukey's post-hoc test)

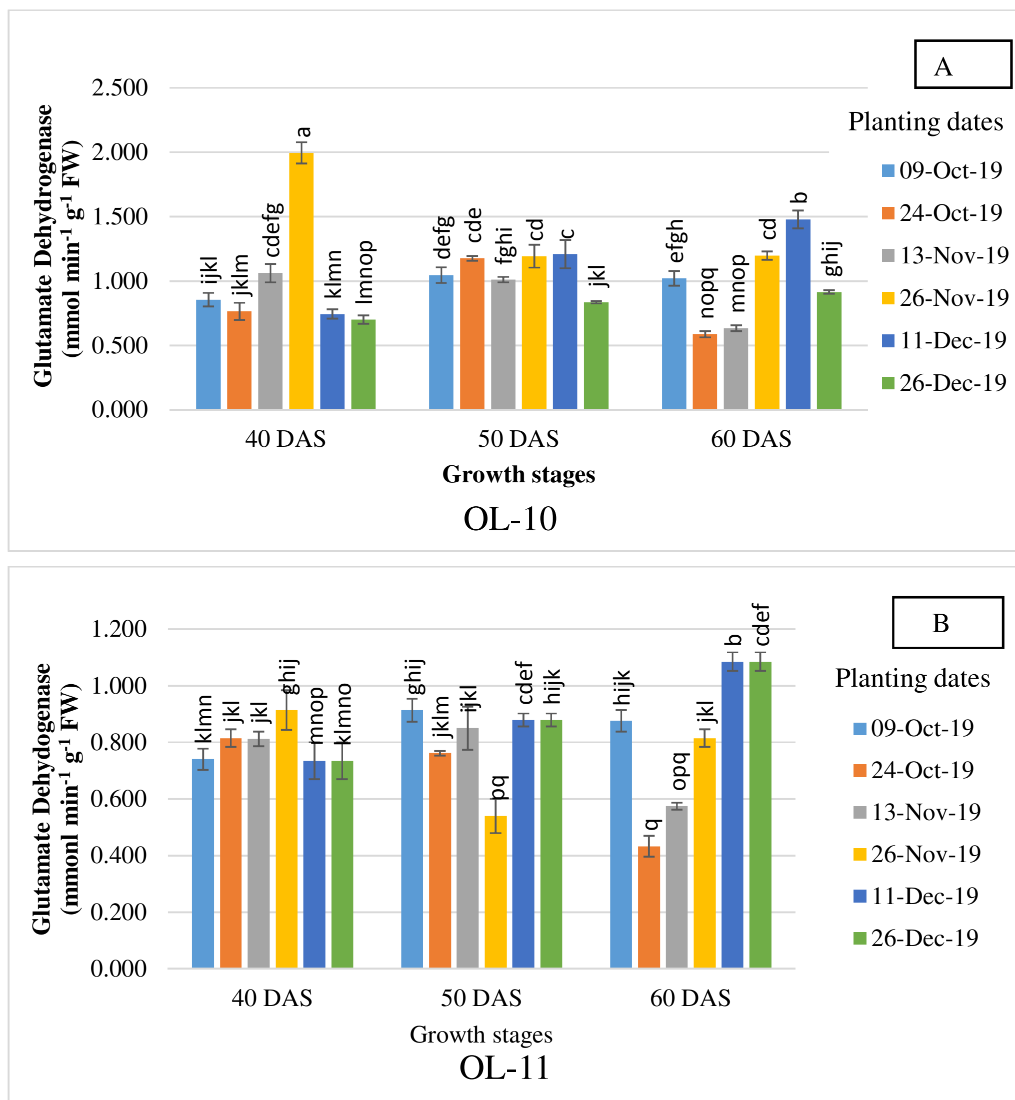


Fig 6 Glutamate dehydogenase activity (mmol min^{-1} g^{-1} FW) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes (Error bars represents standard error)

4.1.6 Glutamate Oxaloacetate Transaminase (GOT)

Glutamate Oxaloacetate Transaminase (GOT) plays a pivotal role in regulating the nitrogen metabolism in crop plants (Liu *et al* 2014). GOT is the key enzyme involved in amino acid degradation alongwith synthesis of new amino acids (Asthir and Tak 2017). The nitrogen is mainly supplied in the form of amino acid and proteins to developing organs of the plants through GOT enzyme activity (Anjana *et al* 2011). GOT activity showed significant (F=1503.1, P<0.01) variation with staggered planting dates and lied in the range from 0.293-1.144 µmol min^{-1} g^{-1} FW (Table 6). Highest GOT activity was observed at Dec 11 planting date and lowest GOT activity was exhibited at Oct 24 planting date. The high amount of

amino acids formed during later planting dates (Nov 26, Dec 11 and Dec 26) may helps in inducing its activity for ammonia assimilation, translocation and reassimilation. The positive correlation of GOT activity with other studied enzymes i.e GS, GOGAT, GDH and GPT revealed that the enzyme is involved in ammonia assimilation.

GOT activity also varied significantly ($F=624.1$, $P<0.01$) among growth stages and lied in the range from 0.597-0.920 µmol min^{-1} g^{-1} FW. GOT activity increased with maturity at almost all the growth stages of respective planting dates from 40 to 50 DAS but declined further. GOT activity was significantly ($F=546.3$, $P<0.01$) high in OL-10 genotype at almost all the planting dates and growth stages in comparison with OL-11, the slow growing single cut variety. Significant differences were reported in wheat genotypes by earlier workers (Kaur *et al* 2017, Asthir and Tak 2017, Asthir *et al* 2018). The three way interaction of planting dates, growth stages and genotypes was found to be significant ($F=29.2$, $P<0.01$).

Table 6 Glutamate Oxaloacetate Transaminase activity (µmol min^{-1} g^{-1} FW) as influenced by planting dates and growth stages in oat genotypes.

Glutamate Oxaloacetate Transaminase (µmol min^{-1} g^{-1} FW)					
Genotype	**Planting dates**	**Growth stages (DAS)**			
		40	**50**	**60**	**Mean**
OL-10	09-Oct-19	0.665±0.03^{hijk}	0.830±0.03^{g}	0.569±0.01^{lmn}	**0.688^{f}**
	24-Oct-19	0.594±0.02^{jklmn}	0.519±0.01^{no}	0.250±0.02^{q}	**0.454^{i}**
	13-Nov-19	0.874±0.01^{fg}	1.221±0.02^{bc}	0.906±0.01^{efg}	**1.000^{b}**
	26-Nov-19	0.511±0.04^{no}	0.920±0.03^{ef}	0.850±0.03^{fg}	**0.760^{e}**
	11-Dec-19	0.885±0.04^{efg}	1.147±0.04^{c}	1.400±0.01^{a}	**1.144^{a}**
	26-Dec-19	0.728±0.04^{h}	0.884±0.03^{efg}	0.653±0.04^{hijkl}	**0.755^{e}**
Mean		**0.710^{d}**	**0.920^{a}**	**0.771^{c}**	
OL-11	09-Oct-19	0.591±0.02^{klmn}	0.709±0.03^{h}	0.259±0.02^{pq}	**0.520^{h}**
	24-Oct-19	0.482±0.02^{o}	0.258±0.02^{qp}	0.139±0.02^{r}	**0.293^{j}**
	13-Nov-19	0.677±0.01^{hij}	1.032±0.04^{d}	0.964±0.03^{de}	**0.891^{cd}**
	26-Nov-19	0.339±0.02^{p}	0.902±0.02^{efg}	0.559±0.04^{mno}	**0.600^{g}**
	11-Dec-19	0.622±0.01^{ijklm}	0.923±0.01^{ef}	1.234±0.01^{b}	**0.926^{c}**
	26-Dec-19	0.868±0.04^{fg}	1.024±0.03^{d}	0.689±0.01^{hi}	**0.860^{d}**
Mean		**0.597^{f}**	**0.808^{b}**	**0.641^{e}**	
CD at 5%	Planting dates (PD) = 0.026 , Growth stages (GS) = 0.015, Genotypes (G) = 0.01, PD x GS = 0.045 , PD x G = 0.042, GS x G = NS, PD x GS x G = 0.050				

Values are mean ± standard deviation of three replications
NS = Non-significant
Values with same letter(s) are non-significant at 5% level of significance (Tukey's post-hoc test)

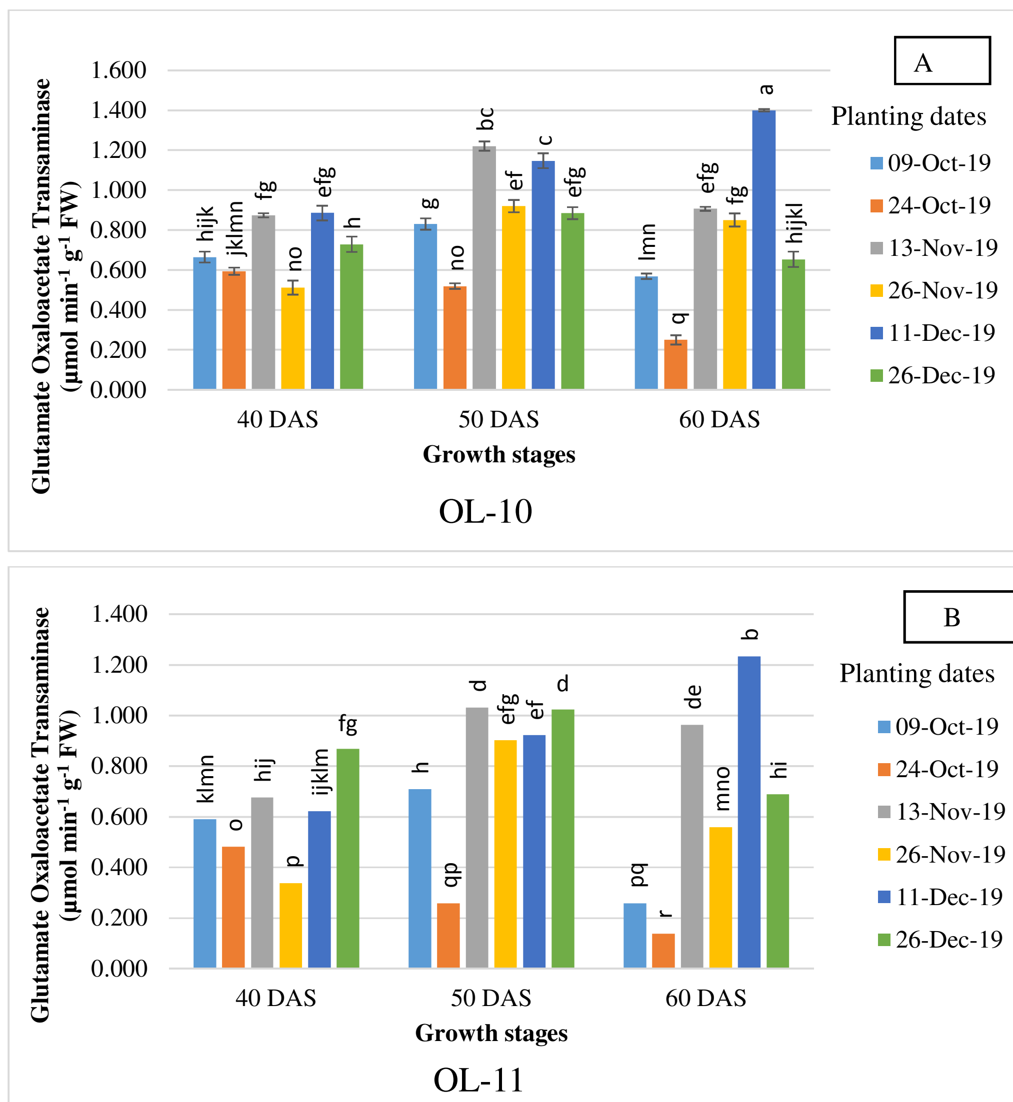


Fig 7 Glutamate Oxaloacetate Transaminase activity (µmol min^{-1} g^{-1} FW) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes (Error bars represents standard error)

4.1.7 Glutamate Pyruvate Transaminase (GPT)

Glutamate Pyruvate Transaminase (GPT) is the key enzyme occupying the central position between N and C metabolism by maintaining the cellular levels of three major components i.e. glutamate, ammonium ions and α-ketoglutarate (Dubois *et al* 2003). GPT activity differed significantly ($F=770.8$, $P<0.01$) among staggered planting dates.The GPT activity lied in the range from 0.337-1.144 µmol min^{-1} g^{-1} FW (Table 7). Highest GPT activity was observed at Nov 13 planting date in OL-10 genotype and Dec 26 planting date in OL-11 genotype. High free amino acid content may be responsible for higher GPT activity during Dec 26 planting date. Kaur *et al* (2017) reported significant increase in GPT activity with increased free amino acid content. In our study, low temperature prevailing during the

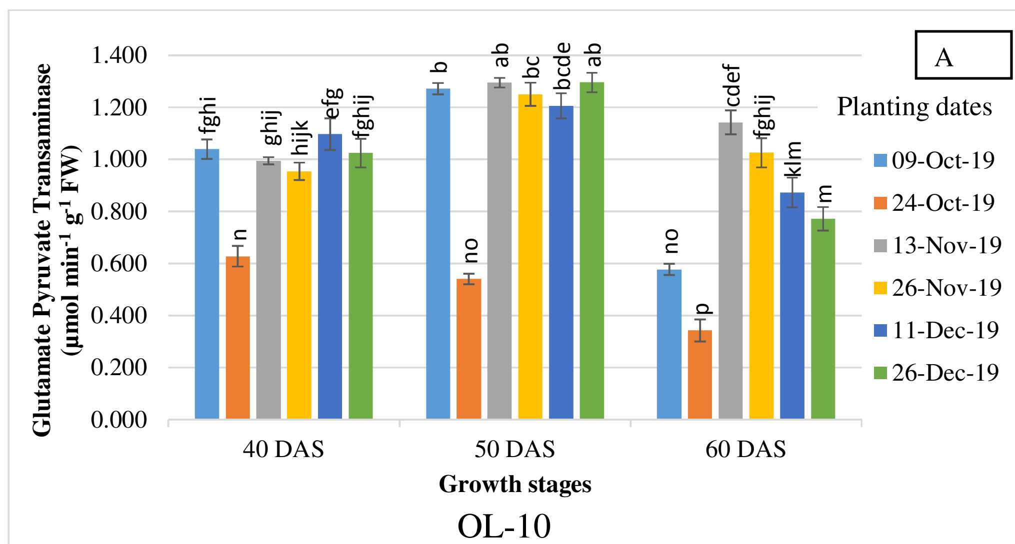
growing period might be responsible for high free amino acid content as well as proteins which ultimately upregulated the GPT activity during later planting dates. The GPT activity showed positive correlation with GS, GOGAT, GDH and GOT enzymes.

GPT activity also varied significantly (F= 807.3, P<0.01) among growth stages. GPT activity increased with maturity from 40 to 50 DAS and declined further at 60 DAS. GPT activity was significantly (F=453.1, P<0.01) higher in OL-10 genotype at almost all the planting dates and growth stages. Genotypic differences were also reported by previous workers in wheat (Kaur *et al* 2017, Asthir and Tak 2017 and Asthir *et al* 2018). The three way interaction of planting dates, growth stages and genotypes was found to be significant (F=12.6, P<0.01).

Table 7 Glutamate Pyruvate Transaminase activity (μmol min^{-1} g^{-1} FW) as influenced by planting dates and growth stages in oat genotypes.

Glutamte Pyruvate Transaminase (μmol min^{-1} g^{-1} FW)					
Genotype	**Planting dates**	**Growth stages (DAS)**			
		40	**50**	**60**	**Mean**
OL-10	09-Oct-19	1.039±0.04^{fghi}	1.271±0.02^{b}	0.577±0.02^{no}	**0.962^{d}**
	24-Oct-19	0.628±0.04^{n}	0.540±0.02^{no}	0.343±0.04^{p}	**0.503^{f}**
	13-Nov-19	0.995±0.01^{ghij}	1.294±0.02^{ab}	1.142±0.05^{cdef}	**1.144^{a}**
	26-Nov-19	0.954±0.03^{hijk}	1.250±0.04^{bc}	1.025±0.06^{fghij}	**1.077^{bc}**
	11-Dec-19	1.097±0.06^{efg}	1.206±0.05^{bcde}	0.872±0.06^{klm}	**1.058^{c}**
	26-Dec-19	1.024±0.05^{fghij}	1.296±0.04^{ab}	0.771±0.05^{m}	**1.030^{c}**
Mean		**0.956^{c}**	**1.143^{a}**	**0.788^{d}**	
OL-11	09-Oct-19	0.911±0.03^{jkl}	1.220±0.02^{bcd}	0.323±0.01^{p}	**0.818^{e}**
	24-Oct-19	0.500±0.03^{o}	0.333±0.03^{p}	0.177±0.02^{q}	**0.337^{g}**
	13-Nov-19	0.591±0.02^{no}	1.063±0.03^{fgh}	0.857±0.03^{klm}	**0.837^{e}**
	26-Nov-19	0.761±0.02^{m}	0.859±0.04^{klm}	0.802±0.05^{lm}	**0.807^{e}**
	11-Dec-19	0.837±0.03^{klm}	1.090±0.05^{efg}	0.941±0.04^{ijk}	**0.956^{d}**
	26-Dec-19	1.118±0.03^{def}	1.391±0.03^{a}	0.868±0.05^{klm}	**1.126^{ab}**
Mean		**0.787^{d}**	**0.993^{b}**	**0.661^{e}**	
CD at 5%	Planting dates (PD) = 0.036, Growth stages (GS) = 0.021 , Genotypes (G) = 0.014, PD x GS = 0.062 , PD x G = 0.058 , GS x G = NS, PD x GS x G = 0.065				

Values are mean ± standard deviation of three replications
NS = Non-significant
Values with same letter(s) are non-significant at 5% level of significance (Tukey's post-hoc test)



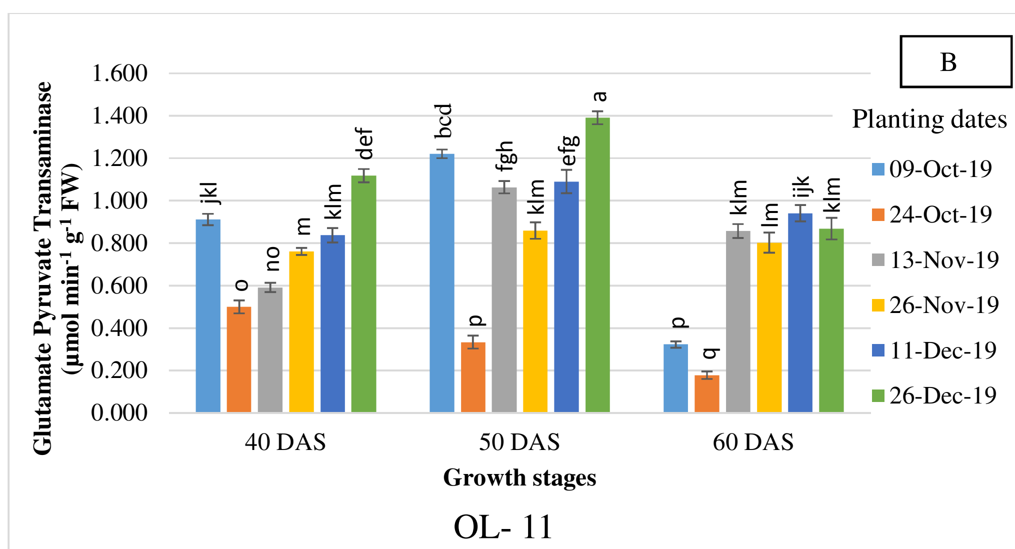


Fig 8 Glutamate pyruvate transaminase activity (µmol min^{-1} g^{-1} FW) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes (Error bars represents standard error)

4.1.8 Chlorophyll content

Chlorophyll content is the marker of photosynthetic efficiency of plants. Temperature is the main factor affecting the photosynthetic capacity thereby altering chlorophyll content. Chlorophyll content differed significantly ($F = 1359.9$, $P<0.01$) with staggered planting dates. Among six planting dates, total chlorophyll content ranged from 0.583-0.955 mg g^{-1} FW (Table 8). Highest chlorophyll content was observed at Oct 9 planting date and lowest was observed at Dec 11 planting date. Chlorophyll content was high during the initial planting dates (Oct 9, Oct 24 and Nov 13) and decreased with delay in planting time. Several workers reported that low temperature prevailing during the growing period disrupt various processes

of photosynthesis like stomatal closure, carbon assimilation and thylakoid electron transport which ultimately reduces the chlorophyll content (Aghee *et al* 2011, Koc *et al* 2010, Dhillon and Uppal 2019). Additionally, less available sunshine hours during later planting dates suppresses the chlorophyll biosynthetic enzymes and also reduces available carbon skeletons resulting in lesser chlorophyll content. The present study is in conformity with Zhao *et al* (2020).

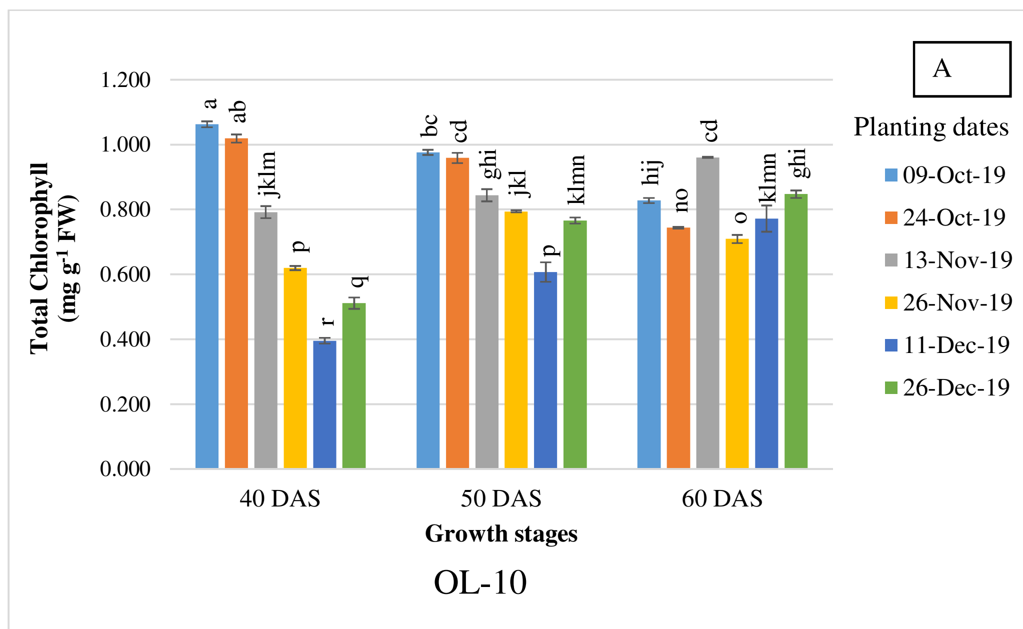
Chlorophyll content also depicted significant (F=521.1, P<0.01) difference with growth stages and lied in the range from 0.671-0.824 mg g^{-1} FW. The chlorophyll content during growth stages varied according to sunshine hours. Significant differences in growth stages were also recorded by previous workers (Kaur *et al* 2015, Zhang *et al* 2009 and Yamamoto *et al* 2002).

Significant (F=404.8, P<0.01) variation was observed for chlorophyll content among two oat genotypes. The photosynthetic pigments in OL-10 may be better protected against low temperature as compared to OL-11 which resulted in higher chlorophyll content in OL-10 genotype. The present study confirmed the findings of previous workers (Kaur *et al* 2015, Devi *et al* 2019). The three way interaction of planting dates, growth stages and genotypes was found to be significant.

Table 8 Total Chlorophyll content (mg/g FW) as influenced by planting dates and growth stages in oat genotypes.

Total Chlorophyll content (mg g^{-1}FW)					
Genotype	**Planting dates**	**Growth stages (DAS)**			**Mean**
		40	**50**	**60**	
OL-10	09-Oct-19	1.063±0.01^{a}	0.976±0.01^{bc}	0.828±0.01^{hij}	**0.955^{a}**
	24-Oct-19	1.019±0.01^{ab}	0.959±0.02^{cd}	0.744±0.00^{no}	**0.907^{b}**
	13-Nov-19	0.792±0.02^{jklm}	0.844±0.02^{ghi}	0.961±0.00^{cd}	**0.866^{c}**
	26-Nov-19	0.619±0.01^{p}	0.794±0.00^{jkl}	0.709±0.01^{o}	**0.708^{f}**
	11-Dec-19	0.396±0.01^{r}	0.607±0.03^{p}	0.772±0.04^{klmn}	**0.592^{h}**
	26-Dec-19	0.512±0.02^{q}	0.766±0.01^{klmn}	0.847±0.01^{ghi}	**0.708^{f}**
Mean		**0.733^{e}**	**0.824^{a}**	**0.810^{b}**	
OL-11	09-Oct-19	0.995±0.01^{bc}	0.926±0.00^{de}	0.749±0.01^{lmno}	**0.890^{b}**
	24-Oct-19	0.885±0.01^{efg}	0.811±0.01^{ijk}	0.643±0.01^{p}	**0.779^{d}**
	13-Nov-19	0.647±0.01^{p}	0.733±0.01^{no}	0.857±0.01^{fgh}	**0.746^{e}**
	26-Nov-19	0.521±0.01^{q}	0.754±0.00^{lmno}	0.639±0.01^{p}	**0.638^{g}**
	11-Dec-19	0.367±0.02^{r}	0.633±0.02^{p}	0.748±0.01^{mno}	**0.583^{h}**
	26-Dec-19	0.610±0.02^{p}	0.819±0.02^{hij}	0.898±0.01^{ef}	**0.776^{d}**
Mean		**0.671^{f}**	**0.779^{c}**	**0.756^{d}**	
CD at 5%	Planting dates (PD) = 0.021, Growth stages (GS) = 0.0173, Genotypes (G) = 0.014, PD x GS = 0.026, PD x G = 0.024, GS x G = 0.021, PD x GS x G = 0.027				

Values are mean ± standard deviation of three replications
NS = Non-significant
Values with same letter(s) are non-significant at 5% level of significance (Tukey's post-hoc test)



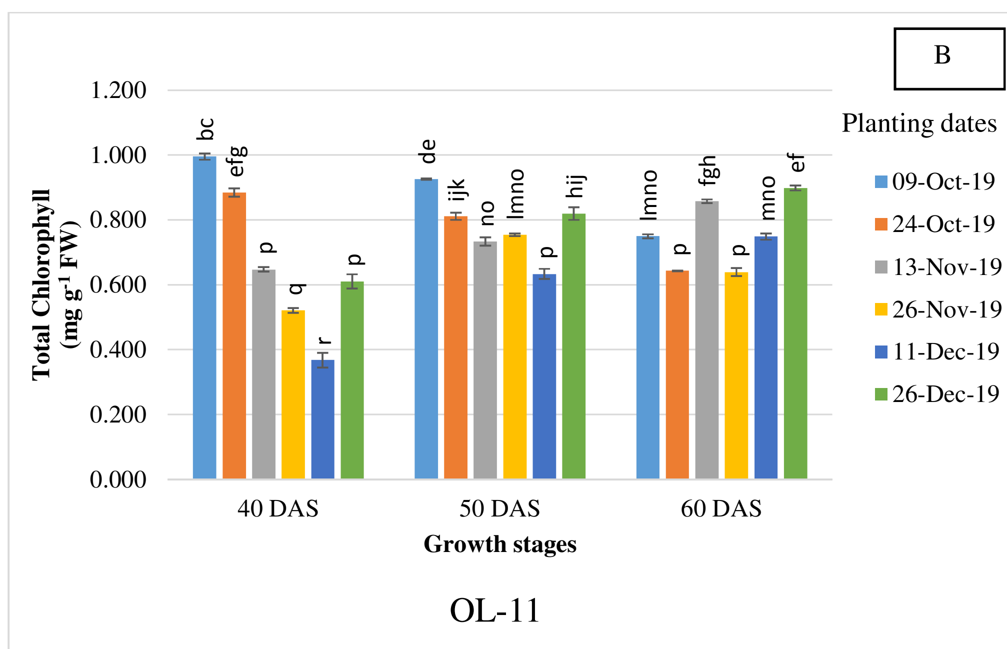


Fig 9 Total Chlorophyll content (mg/g FW) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes (Error bars represents standard error)

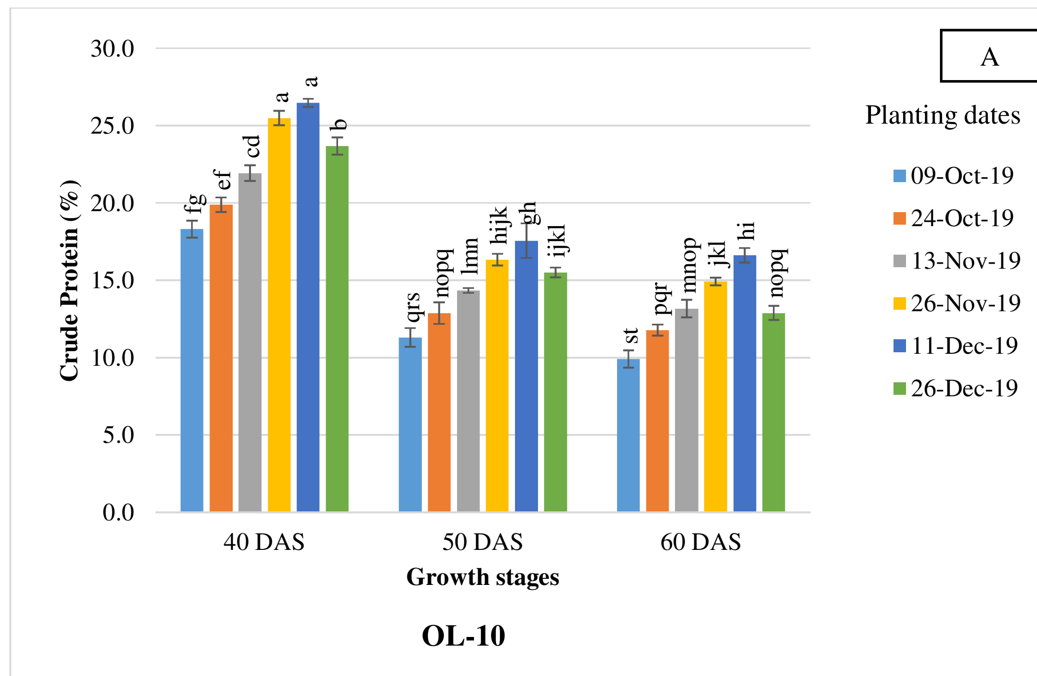
4.2 Effect of different planting dates and growth stages on nutritional composition, digestibility and antinutritional composition in oat genotypes.

4.2.1 Crude protein (CP)

Protein is the primary nutrient found in cereal crops and is likely the principle reason a particular forage is being fed. CP represents the sum of true protein and non-protein crude protein and the ruminants can utilize a portion of both types of protein. CP content depicted

significantly strong relationship with staggered planting dates (F=588.1, P<0.01) and ranged from 12.3-20.5% (Table 9). Highest CP content was exhibited at Dec 11 planting date and minimum at Oct 9 planting date. The temperature was optimum for oat growth during October and early November whereas low temperature during the late November and early December increased the CP content above normal. During the late planting time (Nov 26 and Dec 11), the nitrogen was not utilized properly by root, stem and leaves because of low temperature stress prevailing during the growing period that resulted in higher CP content. The present study is quite in line withresults of Dhillon *et al* (2019) in barley. The temperature was regained during later harvesting stages of last planting date (Dec 26) resulting in somewhat decreased CP content in comparison with Nov 26 and Dec 11 planting dates.

The variation in CP content was also observed with the maturity of the plant. Significant variation was observed for CP content with growth stages (F=3587.6, P<0.01). Among three growth stages, the CP content ranged from 12.5-22.0% (Table 9). The maximum CP content was observed at 40 DAS followed by 50 DAS and 60 DAS. With growth and development of the plant, photosynthates get utilized in higher biomass production resulting in decreased CP content during later growth stages. Kruse *et al* (2008) reported that nitrogen is replaced by fibre and lignin with the advancing age of the crop. The present investigation had shown consistency with previous findings on growth stages with Jehangir *et al* (2012), Hussain *et al* (2004), Malik *et al* (2014) and Bhilare and Joshi (2007) in different forage crops.



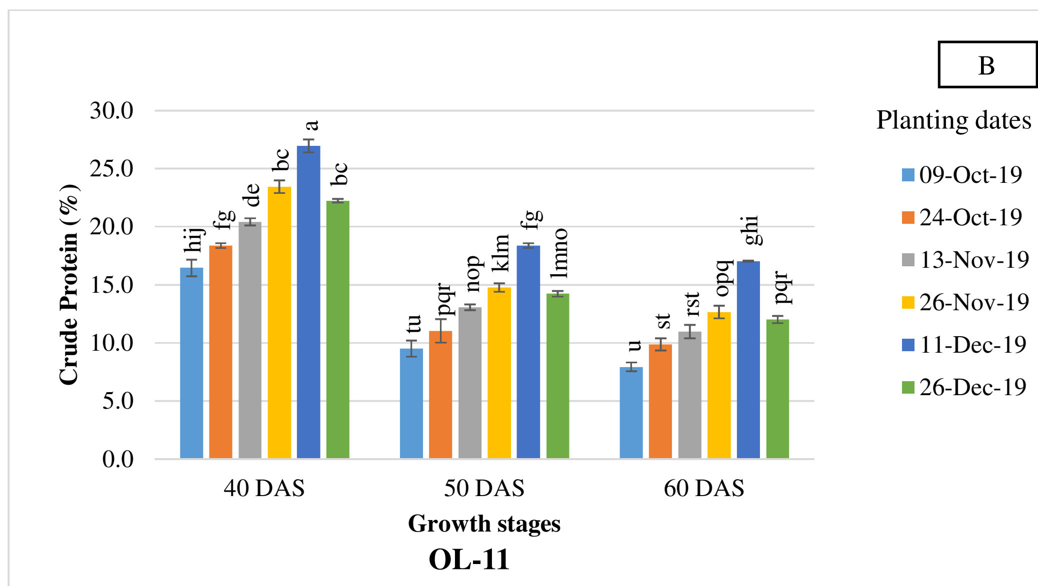


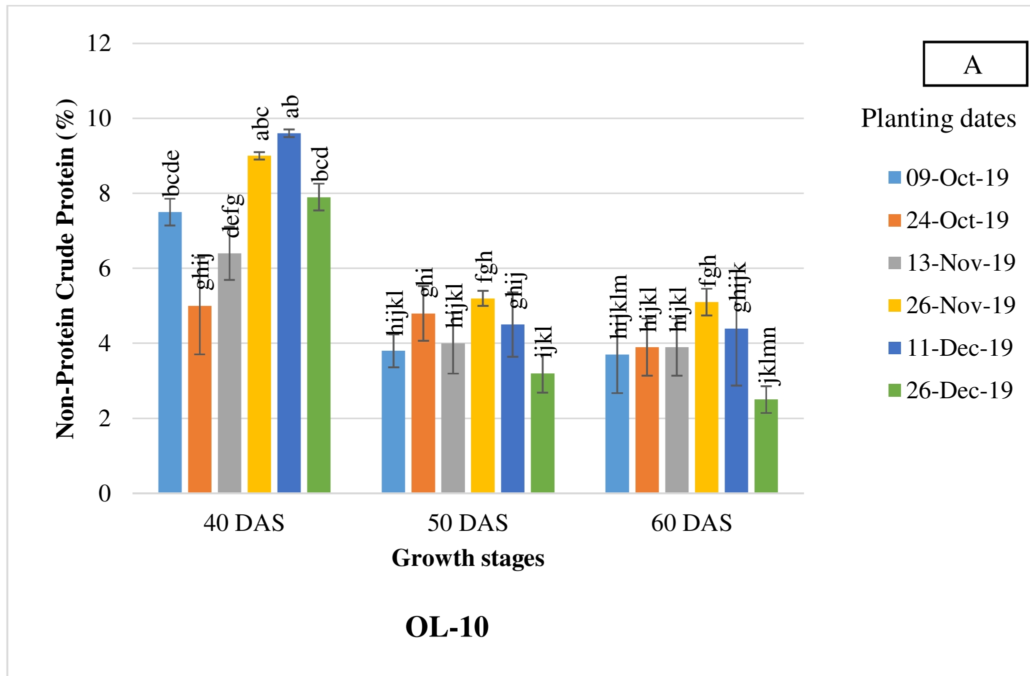
Fig10 Crude protein content (%) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes (Error bars represents standard error)

The mean value of crude protein content (%) of two oat genotypes revealed that the genotype OL-10 (16.9%) had significantly (F=169.84, P<0.01) higher value than OL-11 (15.6%) genotype at almost all the planting dates and growth stages. The high crude protein content genotypes are preferred because they are required for the formation of ruminant's own body proteins and for increasing the milk yield as well (Eskandari *et al* 2009).

4.2.2 Non-protein crude protein (NPCP)

NPCP represents the amount of nitrogen present in the compounds other than proteins such as amines, amides etc. NPCP varied significantly (F=48.4, P<0.01) among staggered planting dates and lied in the range from 4.0-6.3% (Table 9). Highest NPCP content was observed at Dec 11 planting date and lowest at Nov 13 and Dec 26 planting date. The variability in the climatic conditions may be responsible for differential NPCP content with staggered planting.

The NPCP content also differed significantly (F=421.5, P<0.01) with growth stages and lied in the range from 3.3-7.4% (Table 9). Highest NPCP content was exhibited at 40 DAS and further it got declined with the maturity of the plant. Eppendorfer (1971) suggested that NPCP is stored in the vegetative parts of the plant at the expence of true protein.OL-10 (5.2%) had significantly (F= 54.5, P<0.01) higher NPCP content than OL-11 genotype (4.3%). NPCP content is positively correlated (r = 0.866, P<0.01) with crude protein content (Table 21).



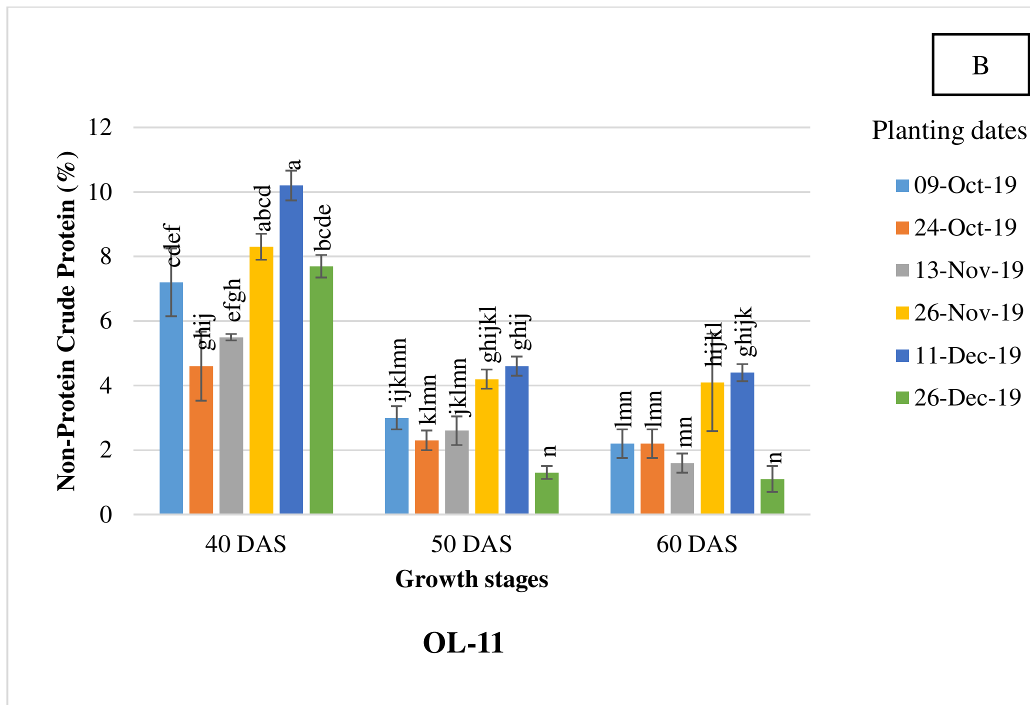


Fig 11 Non-protein crude protein content (%) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes (Error bars represents standard error)

Table 9 Crude protein and Non-protein crude protein content as influenced by planting dates and growth stages in oat genotypes (Percent, Dry matter basis).

PLANTING DATE	GROWTH STAGE (DAS)	Crude protein (%)		Non-protein crude protein (%)	
		Genotypes			
		OL-10	OL-11	OL-10	OL-11
09-Oct-19	Cut at 40 DAS	18.3±0.55	16.5±0.70	7.5±0.36	7.2±1.05
	Cut at 50 DAS	11.3±0.60	9.5±0.70	3.8±0.44	3.0±0.36
	Cut at 60 DAS	9.9±0.55	7.9±0.38	3.7±1.03	2.2±0.44
24-Oct-19	Cut at 40 DAS	19.9±0.46	18.4±0.2	5.0±1.29	4.6±1.07
	Cut at 50 DAS	12.9±0.70	11.8±1.01	4.8±0.73	2.3±0.30
	Cut at 60 DAS	11.8±0.36	9.9±0.52	3.9±0.76	2.2±0.44
13-Nov-19	Cut at 40 DAS	21.9±0.51	20.4±0.31	6.4±0.71	5.5±0.10
	Cut at 50 DAS	14.4±0.15	13.1±0.25	4.0±0.81	2.6±0.44
	Cut at 60 DAS	13.2±0.56	11.0±0.57	3.9±0.76	1.6±0.30
26-Nov-19	Cut at 40 DAS	25.5±0.46	23.5±0.55	9.0±0.10	8.3±0.40
	Cut at 50 DAS	16.3±0.38	14.8±0.36	5.2±0.20	4.2±0.30
	Cut at 60 DAS	14.9±0.25	12.7±0.55	5.1±0.36	4.1±1.51
11-Dec-19	Cut at 40 DAS	26.5±0.26	27.0±0.55	9.6±0.10	10.2±0.46
	Cut at 50 DAS	17.6±1.12	18.4±0.20	4.5±0.86	4.6±0.30
	Cut at 60 DAS	16.6±0.47	17.0±0.04	4.4±1.53	4.4±0.27
26-Dec-19	Cut at 40 DAS	23.7±0.56	22.2±0.15	7.9±0.36	7.7±0.35
	Cut at 50 DAS	15.5±0.31	14.2±0.25	3.2±0.51	1.3±0.20
	Cut at 60 DAS	12.9±0.46	12.0±0.31	2.5±0.36	1.1±0.40
Mean					
Planting date (PD)	09-Oct-19	12.3^{f}		4.6^{b}	
	24-Oct-19	14.1^{e}		3.8^{c}	
	13-Nov-19	15.7^{d}		4.0^{bc}	
	26-Nov-19	18.0^{b}		6.0^{a}	
	11-Dec-19	20.5^{a}		6.3^{a}	
	26-Dec-19	16.8^{c}		4.0^{bc}	
Growth Stages (GS)	Cut at 40 DAS	22.0^{a}		7.4^{a}	
	Cut at 50 DAS	14.2^{b}		3.6^{b}	
	Cut at 60 DAS	12.5^{c}		3.3^{b}	
Genotypes (G)	OL-10	16.9^{a}		5.2^{a}	
	OL-11	15.6^{b}		4.3^{b}	
CD at 5%		PD = 0.496, GS = 0.287, G = 0.195, PD x GS = 0.867, PD x G = 0.810, GS x G =NS, PD x GS x G = NS		PD = 0.926 , GS = 0.756 , G = 0.630 , PD x GS = 1.144 , PD x G = 1.068 , GS x G = 0.926 , PD x GS x G = NS	

Values are mean ± standard deviation of three replications
NS = Non-significant
Values with same letter(s) are non-significant at 5% level of significance (Tukey's post-hoc test)

4.2.3 True Protein

True protein represents the nitrogen present in the protein part of the sample. Significant relationship (F=321.8, $P<0.01$) was found for true protein content among staggered planting dates and lied in the range from 7.7-14.3% (Table 10). Highest true protein content was observed at Dec 11 planting date and lowest at Oct 9 planting date. The increased true proteins with delay in planting was because of seasonal variations, most predominantly

the decline in temperature during the crop life cycle. Earlier study reported that the protein content got uplifted with low temperature stress (Goyal and Kaur 2018). Proteins were known to play a protective role against adverse environmental conditions such as it can prevent oxidative damage to plants (Karimzadeh *et al* 2006). Moreover, it was also observed in a previous study that protein content increased from early to late sowing in wheat (Singh *et al* 2012)

True protein content decreased significantly (F=978.2, P<0.01) with the maturity of the plant lied in the range from 9.2-14.6%. Highest true protein was found at 40 DAS and lowest at 60 DAS.The availability of nutrients start declining because of dilution effect with advancing age of vegetative fractions of plants which may be the reason for decreased true proteins from 40-60 DAS. True protein content was non-significant among two genotypes. The true protein content was 11.6% in OL-10 genotype and 11.2% in OL-11 genotype.

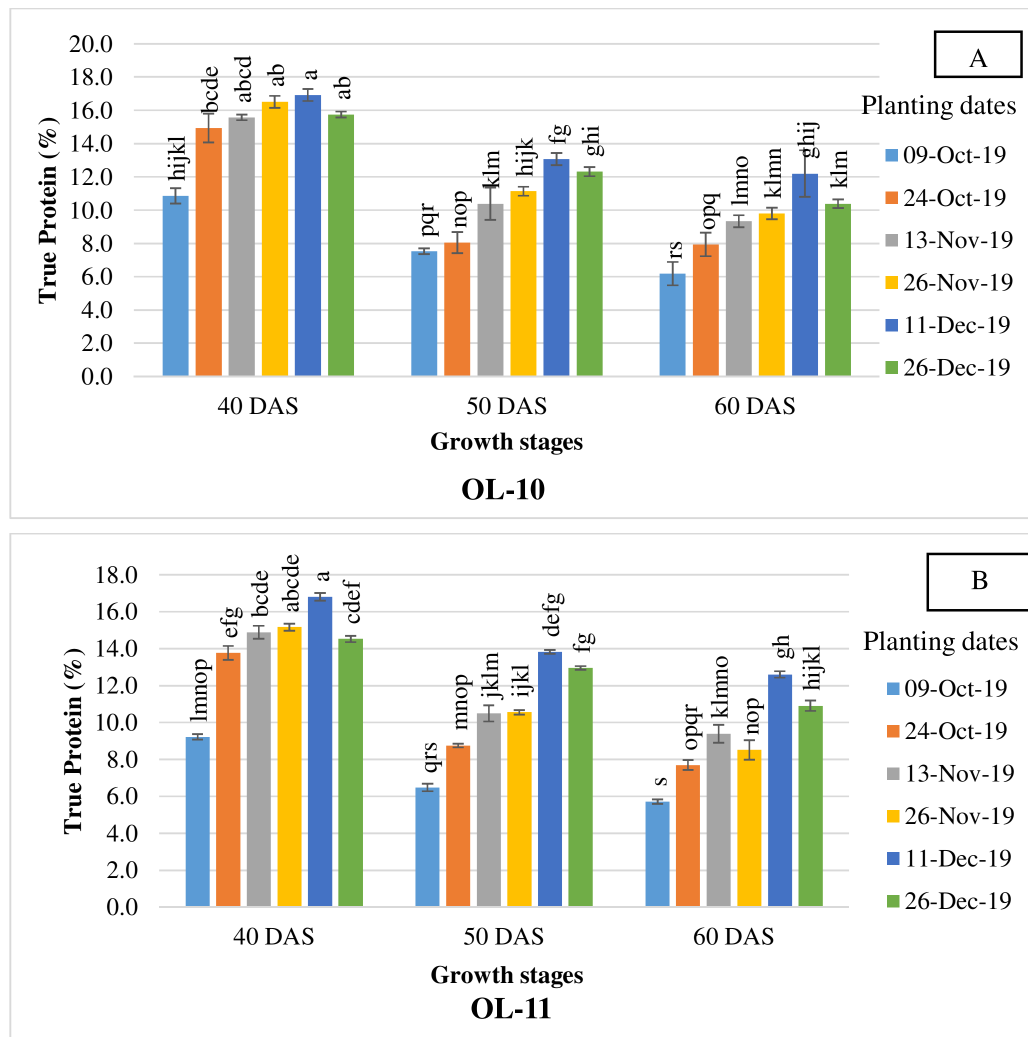


Fig 12 True protein content (%) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes (Error bars represents standard error)

4.2.4 Free amino acids

Free amino acids are considered nitrogen remobilizable pools which monitors the nitrogen status of the plant (Martinez-Andujar *et al* 2013). Significant effect (F=305.4, P<0.01) of staggered planting dates was observed for free amino acid content. Among six planting dates, the free amino acid content ranged from 1.58-3.79 mg g^{-1} DW (Table 10). Data table depicted that highest free amino acid content was exhibited at Dec 26 planting date and lowest was exhibited at Oct 9 planting date. The increased amino acid content is directly associated with GPT and GOT activity which were observed high during later planting dates.

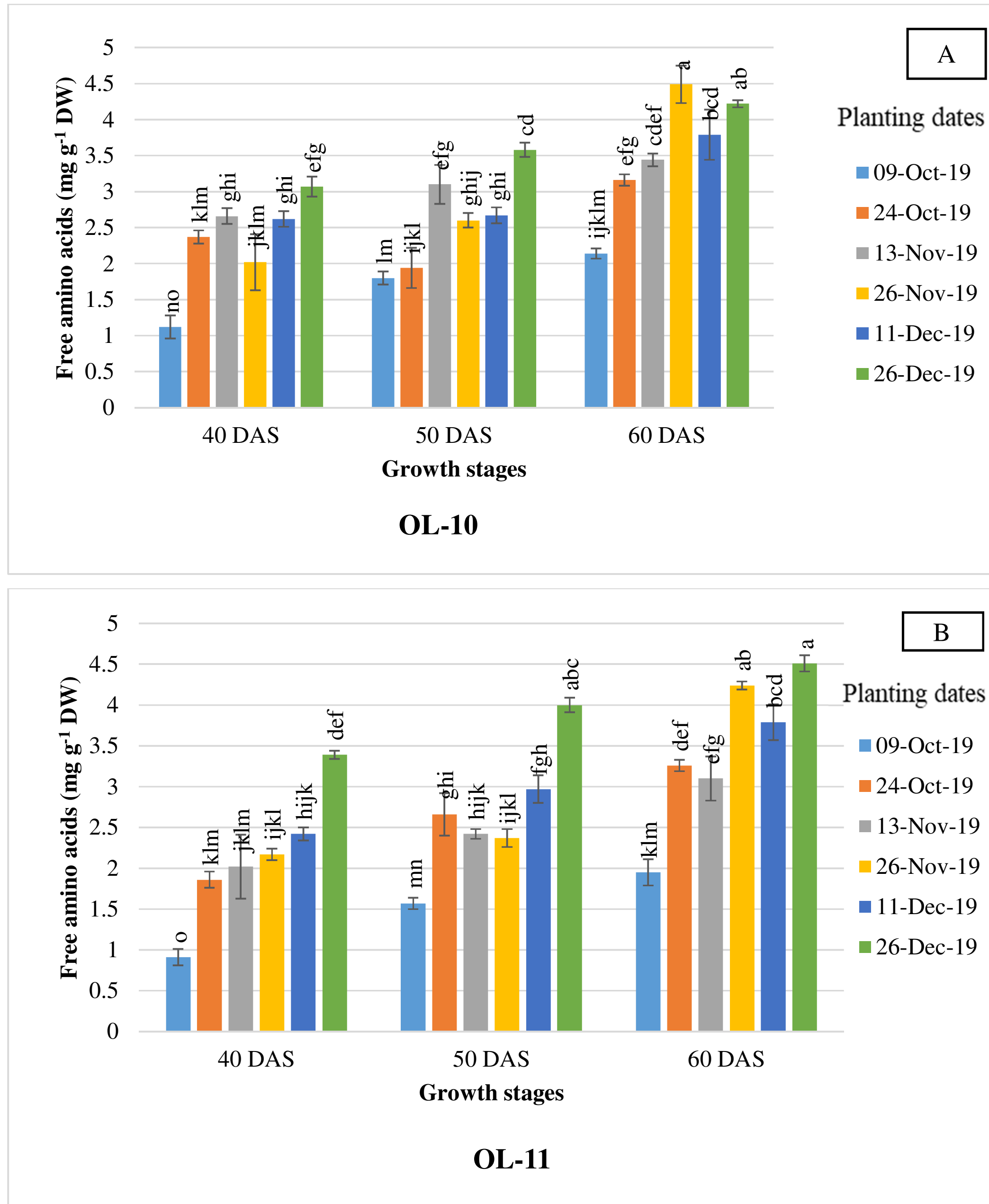


Fig 13 Free amino acids content (mg/g DW) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes (Error bars represents standard error)

Table 10 True protein (Percent, Dry matter basis) and free amino acids (mg/g, Dry matter basis) as influenced by planting dates and growth stages in oat genotypes.

PLANTING DATE	GROWTH STAGE (DAS)	True Protein (%)		Free amino acids (mg g^{-1}DW)	
		Genotypes			
		OL-10	OL-11	OL-10	OL-11
09-Oct-19	Cut at 40 DAS	10.9±0.46	9.2±0.15	1.12±0.16	0.91±0.08
	Cut at 50 DAS	7.5±0.18	6.5±0.20	1.80±0.09	1.57±0.07
	Cut at 60 DAS	6.2±0.71	5.7±0.12	2.14±0.07	1.95±0.16
24-Oct-19	Cut at 40 DAS	14.9±0.86	13.8±0.38	1.94±0.28	1.86±0.1
	Cut at 50 DAS	8.1±0.63	8.8±0.10	2.37±0.09	2.66±0.26
	Cut at 60 DAS	7.9±0.71	7.7±0.27	3.16±0.08	3.26±0.07
13-Nov-19	Cut at 40 DAS	15.6±0.17	14.9±0.35	2.66±0.11	2.02±0.39
	Cut at 50 DAS	10.4±0.96	10.5±0.44	3.10±0.27	2.42±0.06
	Cut at 60 DAS	9.3±0.36	9.4±0.49	3.44±0.09	3.10±0.27
26-Nov-19	Cut at 40 DAS	16.5±0.36	15.2±0.19	2.02±0.39	2.17±0.07
	Cut at 50 DAS	11.1±0.27	10.6±0.12	2.60±0.10	2.37±0.11
	Cut at 60 DAS	9.8±0.35	8.5±0.52	4.49±0.26	4.24±0.05
11-Dec-19	Cut at 40 DAS	16.9±0.36	16.8±0.20	2.62±0.11	2.42±0.08
	Cut at 50 DAS	13.1±0.36	13.8±0.10	2.67±0.11	2.97±0.17
	Cut at 60 DAS	12.2±1.40	12.6±0.18	3.79±0.35	3.79±0.22
26-Dec-19	Cut at 40 DAS	15.8±0.17	14.5±0.18	3.07±0.14	3.39±0.05
	Cut at 50 DAS	12.3±0.27	13.0±0.10	3.58±0.10	4.00±0.09
	Cut at 60 DAS	10.4±0.27	10.9±0.29	4.22±0.05	4.51±0.10
Mean					
Planting date (PD)	09-Oct-19	7.7^{e}		1.58^{e}	
	24-Oct-19	10.2^{d}		2.54^{d}	
	13-Nov-19	11.7^{c}		2.79^{c}	
	26-Nov-19	12.0^{c}		2.98^{b}	
	11-Dec-19	14.3^{a}		3.04^{b}	
	26-Dec-19	12.8^{b}		3.79^{a}	
Growth Stages (GS)	Cut at 40 DAS	14.6^{c}		2.18^{c}	
	Cut at 50 DAS	10.5^{b}		2.68^{b}	
	Cut at 60 DAS	9.2^{a}		3.51^{a}	
Genotypes (G)	OL-10	11.6^{a}		2.82^{a}	
	OL-11	11.2^{a}		2.76^{a}	
CD at 5%		PD = 0.743 , GS = 0.607, G = NS, PD x GS = 0.918 , PD x G = 0.856 , GS x G = 0.743 , PD x GS x G = NS		PD = 0.173, GS = 0.100, G = NS, PD x GS = 0.301, PD x G = 0.280, GS x G = 0.173, PD x GS x G = 0.313	

Values are mean ± standard deviation of three replications
NS = Non-significant
Values with same letter(s) are non-significant at 5% level of significance (Tukey's post-hoc test)

In addition, the variability in climatic conditions also resulted in differential free amino acid content during staggered planting. A previous researcher claimed variation in free amino acid content in response to different temperature conditions during growing period in oat (Goyal and Kaur 2018).

The free amino acids were significantly influenced (F=518.4, P<0.01) with the growth stages and ranged from 2.18-3.51 mg g^{-1} DW. Highest free amino acid content was found at 60 DAS and lowest was observed at 40 DAS. The decreased protein content may be responsible for increased free amino acids during later growth stages. Asthir *et al* (2018) also observed significant differences with stages of harvest in wheat.

The free amino acid content varied non-significantly among two genotypes. The free amino acid content was 2.82 mg g^{-1} DW in OL-10 genotype and 2.76 mg g^{-1} DW in OL-11 genotype. But Kaur *et al* (2015) and Kaur and Goyal (2017) reported significant differences among genotypes for free amino acid content. The three-way interaction of planting dates, growth stages and genotypes was significant (F=3.79, P<0.01).

4.2.5 Ash

Ash content is an estimate of total amount of minerals present within the plant tissue and is required for the formation of bones in ruminant animals. The amount of ash varied significantly (F=204.3, P<0.01) with staggered planting dates that ranged from 9.5-12.2% (Table 11). Highest ash content was exhibited at Oct 24 planting date which was statistically at par with Oct 9 planting dateand lowest ash content was observed at Dec 11 planting date. The higher ash content during the initial planting dates might be due to optimum temperature, less moisture and more dry matter content. The ash content got declined with late planting because of adverse weather conditions. The current investigation is in conformity with results of Kadam *et al* (2019) in oat.

Ash content in relation to different growth stages (F=487.5, P<0.01) also depicted significant relationship and ranged between 10.0-12.3% among three growth stages. Highest ash content was observed at 40 DAS followed by 50 DAS and 60 DAS. Similar results were reported by Kaur and Goyal (2017) in oats and Thavaprakaash *et al* (2008) in baby corn.In the current investigation, it was observed that OL-10 genotype (11.2%) contained significantly (F=35.21, P<0.01) higher ash content as compared to OL-11 genotype (10.8%). This might be due to variation in absorption of minerals from soil by thetwo genotypes as well as their utilization within the different organs of the plant. Genotypic variation in ash content was also reported by Ahmed and Hassan (2015) in wheat.

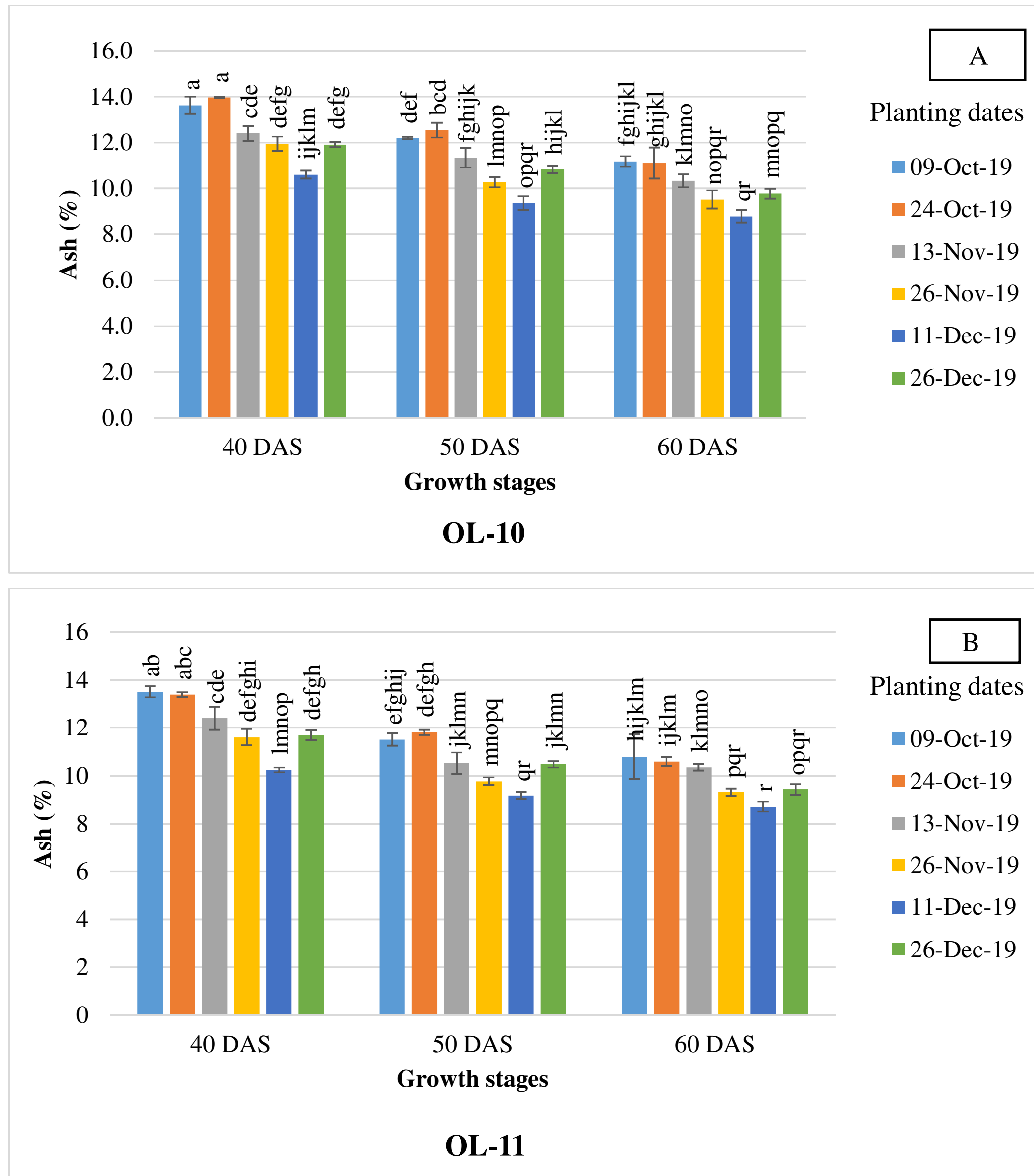


Fig 14 Ash content (%) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes (Error bars represents standard error)

4.2.6 Crude fat (CF)

CF is composed of unsaturated fatty acids, waxes, oil soluble pigments, free fatty acids, cholesterol and phospholipids. It is chiefly required for the maintenance of body functions as well as for milk production in ruminants. Significant ($F=223.5$, $P<0.01$) variation was observed for CF content among staggered planting dates and ranged from 2.3-3.8% (Table 11). Highest crude fat content was observed at Oct 9 planting date and lowest crude fat content was exhibited at Dec 11 planting date. Higher CF content in Oct 9, Oct 24 and Nov 13 sown oat might be due to prevalent environmental conditions required for rapid metabolic activities which enhances the respiration process, thereby converting most of the

carbohydrates into fats. The present investigation reported similar trend with the earlier studies in oat (Kumar 2012, Dar *et al* 2014, Jehangir *et al* 2017) and sorghum (Kumar *et al* 2018).

CF also varied significantly (F=332.7, P<0.01) with growth stages and lied in the range from 2.62-3.55%. Highest crude fat content was exhibited at 50 DAS and lowest at 60 DAS. The decline in CF content with the maturity of the plant may due to the fact that plants were at the late vegetative stage and fats were almost utilized for the growth of the plants (Kumar 2012). The OL-10 genotype (3.2%) had significantly (F=28.9, P<0.01) higher CF content in comparison with OL-11 (3.0%). The variability among genotypes was because of differences in the capacity of accumulation of fats by the growing tissues of plants. Genotypic differences for CF content was also reported by Kaur *et al* in barley (2013).

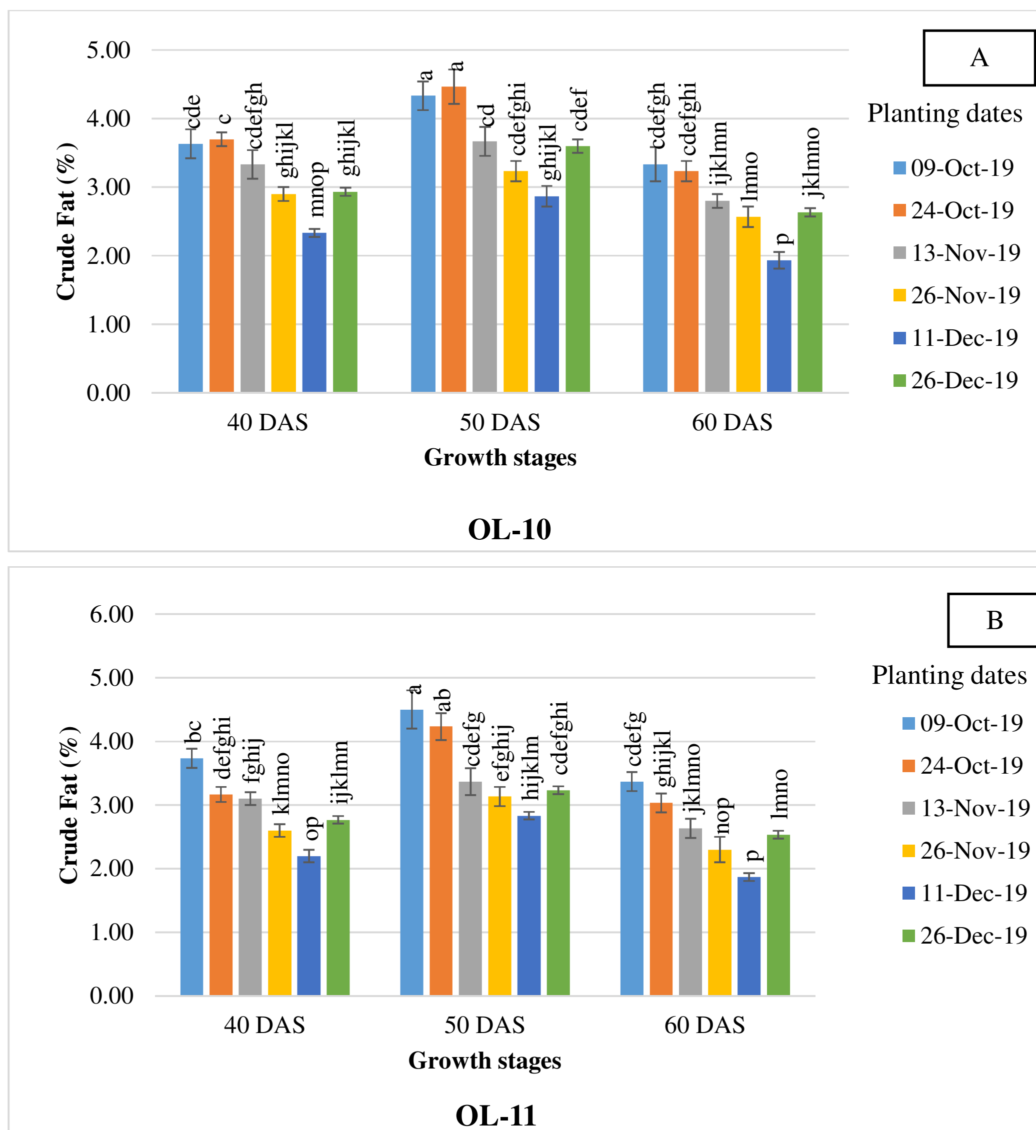


Fig 15 Crude fat content (%) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes (Error bars represents standard error)

Table 11 Crude fat and ash content as influenced by planting dates and growth stages in oat genotypes (Percent, Dry matter basis).

PLANTING DATE	GROWTH STAGE (DAS)	Crude Fat (%)		ASH (%)	
		Genotypes			
		OL-10	OL-11	OL-10	OL-11
09-Oct-19	Cut at 40 DAS	3.63±0.21	3.73±0.15	13.6±0.38	13.5±0.23
	Cut at 50 DAS	4.33±0.21	4.50±0.30	12.2±0.05	11.5±0.25
	Cut at 60 DAS	3.33±0.25	3.37±0.15	11.2±0.22	10.8±0.94
24-Oct-19	Cut at 40 DAS	3.70±0.10	3.17±0.12	14.0±0.02	13.4±0.10
	Cut at 50 DAS	4.47±0.25	4.23±0.21	12.5±0.32	11.8±0.11
	Cut at 60 DAS	3.23±0.15	3.03±0.15	11.1±0.68	10.6±0.18
13-Nov-19	Cut at 40 DAS	3.33±0.21	3.10±0.10	12.4±0.32	12.4±0.49
	Cut at 50 DAS	3.67±0.21	3.37±0.21	11.3±0.43	10.5±0.45
	Cut at 60 DAS	2.80±0.10	2.63±0.15	10.3±0.28	10.4±0.13
26-Nov-19	Cut at 40 DAS	2.90±0.10	2.6±0.10	11.9±0.31	11.6±0.34
	Cut at 50 DAS	3.23±0.15	3.13±0.15	10.3±0.22	9.8±0.17
	Cut at 60 DAS	2.57±0.15	2.30±0.20	9.5±0.39	9.3±0.15
11-Dec-19	Cut at 40 DAS	2.33±0.06	2.20±0.10	10.6±0.18	10.3±0.1
	Cut at 50 DAS	2.87±0.15	2.83±0.06	9.4±0.29	9.2±0.15
	Cut at 60 DAS	1.93±0.12	1.87±0.06	8.8±0.28	8.7±0.20
26-Dec-19	Cut at 40 DAS	2.93±0.06	2.77±0.06	11.9±0.11	11.7±0.21
	Cut at 50 DAS	3.60±0.10	3.23±0.06	10.8±0.17	10.5±0.13
	Cut at 60 DAS	2.63±0.06	2.53±0.06	9.8±0.21	9.4±0.23
Mean					
Planting date (PD)	09-Oct-19	3.8[a]		12.1[a]	
	24-Oct-19	3.6[b]		12.2[a]	
	13-Nov-19	3.2[c]		11.2[b]	
	26-Nov-19	2.8[e]		10.4[c]	
	11-Dec-19	2.3[f]		9.5[d]	
	26-Dec-19	3.0[d]		10.7[c]	
Growth Stages (GS)	Cut at 40 DAS	3.0[b]		12.3[a]	
	Cut at 50 DAS	3.6[a]		10.8[b]	
	Cut at 60 DAS	2.7[c]		10.0[c]	
Genotypes (G)	OL-10	3.2[a]		11.2[a]	
	OL-11	3.0[b]		10.8[b]	
CD at 5%		PD = 0.152, GS = 0.088, G = 0.060, PD x GS = 0.266, PD x G = 0.248, GS x G = NS, PD x GS x G = NS		PD = 0.307, GS = 0.177, G = 0.121, PD x GS = 0.537, PD x G = NS, GS x G =0.307, PD X GS X G = NS	

Values are mean ± standard deviation of three replications
NS = Non-significant
Values with same letter(s) are non-significant at 5% level of significance (Tukey's post-hoc test)

4.2.7 Acid detergent fibre (ADF)

Acid detergent fibre represents percentage of the plant material that consists of cellulose, lignin and silica. High quality fodder crops usually have an ADF content of 25-35%. The significant (F=154.1, P<0.01) effect was found for ADF content among staggered planting dates which ranged from 21.6-33.7% (Table 12). Highest ADF content was exhibited

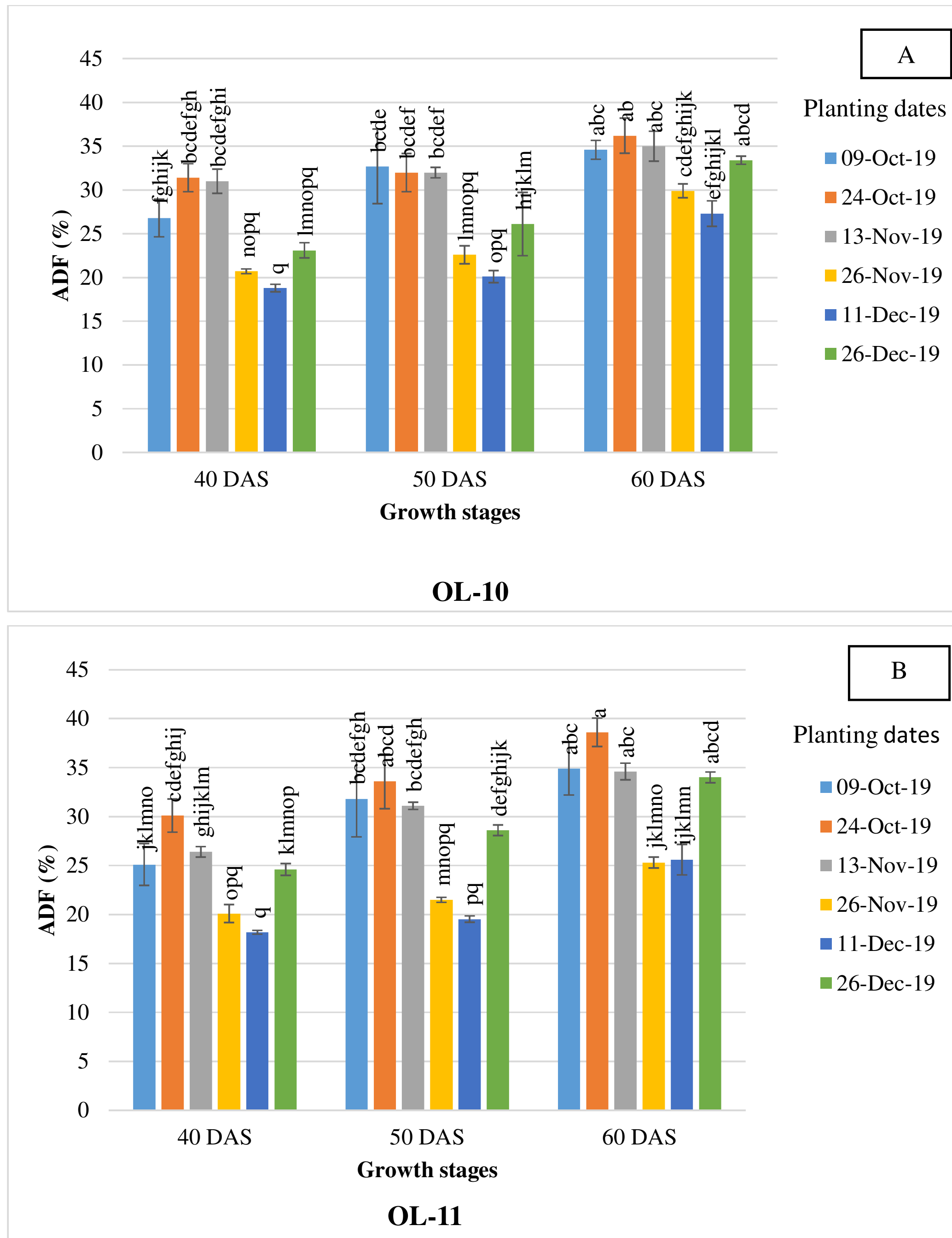


Fig 16 Acid detergent fibre content (%) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes (Error bars represents standard error)

at Oct 24 planting date and lowest ADF content was observed at Dec 11 planting date. The significantly higher ADF content during the initial planting dates might be due to more plant height, less succulency and more thickness of the stem as compared to Nov 26 and Dec 11 planting date. Optimum temperature available for fibre synthesis during October and early November may be responsible for higher fibre content during initial planting dates. The increase in ADF content during the Dec 26 planting date might bedue to increase in temperature as well as sunshine hours required for the formation of fibrous stem. Similar results were reported by Salama and Nawar (2016) in forage maize.

ADF content increased significantly ($F=202.4$, $P<0.01$) with delaying the cutting time and ranged from 24.7-32.5%. The mean values showed that highest ADF content was observed at 60 DAS and lowest at 40 DAS. The fibre fractions increased with advancing maturity because of enhancement of leaf toughness as well as stem thickness that resulted from accrual of more cell wall carbohydrates (Jensen *et al* 2005). The present study is in agreement with previous workers (Kaur and Goyal 2017, Firdous and Gilani 2001 and Firdous and Gilani 1998). The ADF content in OL-10 genotype (28.5%) was non-signifiacnt with OL-11 genotype (28.0%).

4.2.8 Neutral detergent fibre (NDF)

Neutral detergent fibre represents the fibrous bulk of the forage and it consists of cellulose, hemicellulose and lignin which are categorized as plant cell wall or structural carbohydrates. Significant ($F=158.7$, $P<0.01$) relationship was found for NDF contents among staggered planting dates and ranged from 48.6-58.4% (Table 12). The mean values depicted that highest NDF content was found at Oct 24 planting date and lowest NDF content was found at Dec 11 planting date. The higher NDF content during Oct 9, Oct 24 and Nov 13 planting dates was because of favourable climate that resulted in enhanced cell wall carbohydrate synthesis as compared to Nov 26 and Dec 11 planting date. Earlier study reported similar decrease in NDF content in barley with delay in planting time (Dhillon *et al* 2019). The somewhat increased NDF content at Dec 26 planting date might be due to warm climatic conditions which increased the growth of stem.

NDF content increased significantly ($F=385.3$, $P<0.01$) with the advancing maturity of vegetative fractions of the plant and ranged from 49.7-58.8%. In the present study, highest NDF content was observed at 60 DAS and lowest at 40 DAS. The lignification of the cell wall increased with the maturation of the crop which might be the reason for higher NDF content at 60 DAS. Similar results were reported by Bhilare and Joshi (2007) in oats and Firdous and Gilani (2001) in sorghum. The significantly ($F=7.72$, $P<0.01$) higher NDF content was observed in OL-10 genotype (54.7%) at almost all planting dates and growth stages in comparison with OL-11 genotype (53.9%). This was because of differential plant height in both genotypes that resulted in variability in cell wall maturation. Genotypic

differences were also observed by Mut *et al* (2016) in oat. The three way interaction of planting dates, growth stages and genotypes was found to be significant.

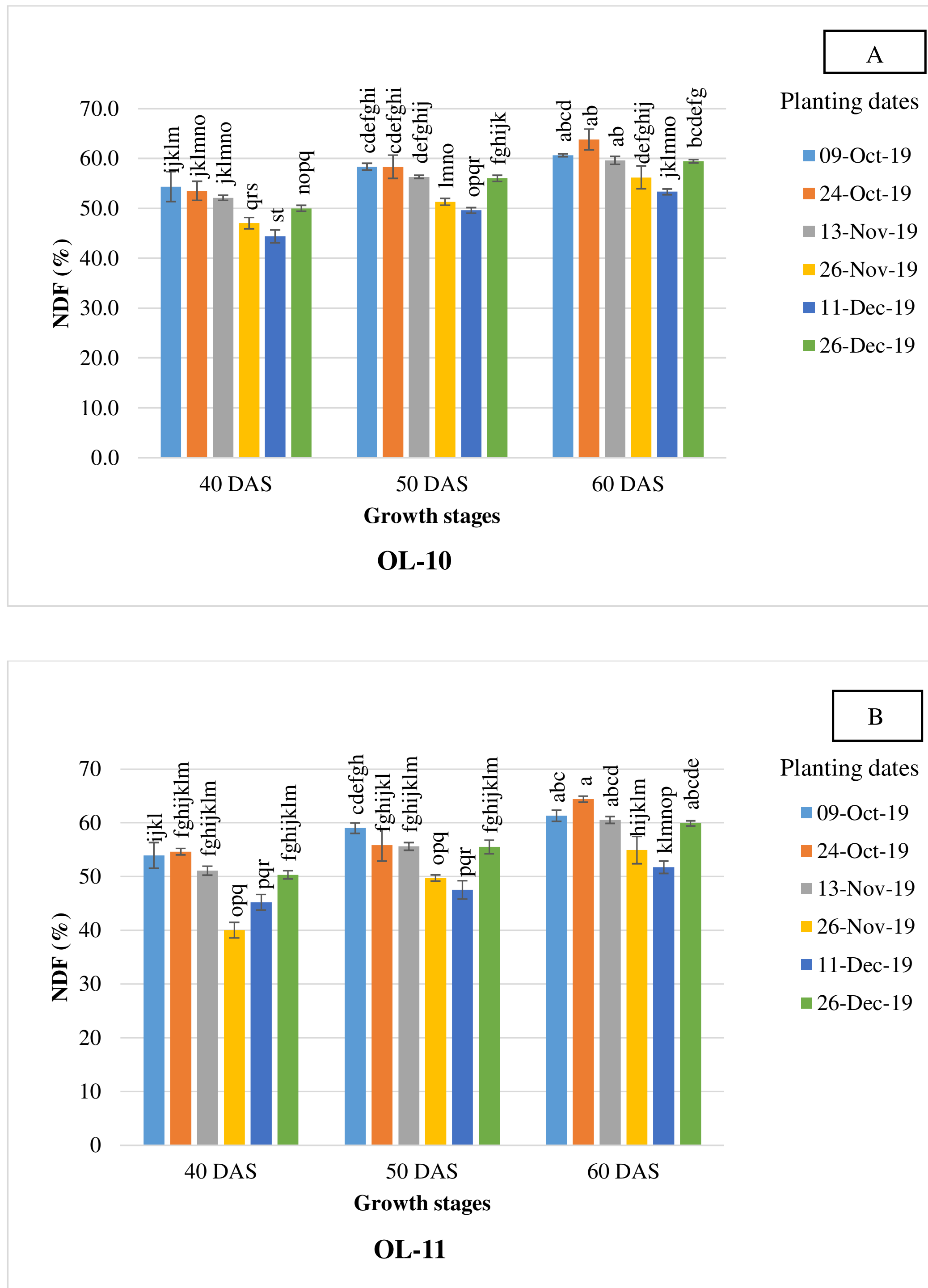


Fig 17 Neutral detergent fibre content (%) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes (Error bars represents standard error)

Table 12 Acid detergent fibre and neutral detergent fibre as influenced by planting dates and growth stages in oat genotypes (Percent, Dry matter basis).

PLANTING DATES	GROWTH STAGES (DAS)	ADF (%)		NDF (%)	
		Genotypes			
		OL-10	OL-11	OL-10	OL-11
09-Oct-19	Cut at 40 DAS	26.8±2.2	25.1±2.1	54.3±3.0	53.9±2.4
	Cut at 50 DAS	32.7±4.3	31.8±3.9	58.3±0.7	59.0±1.0
	Cut at 60 DAS	34.6±1.1	34.9±2.7	60.6±0.3	61.3±1.0
24-Oct-19	Cut at 40 DAS	31.4±1.6	30.1±1.7	53.5±1.9	54.6±0.6
	Cut at 50 DAS	32.0±2.2	33.6±2.8	58.3±2.3	55.8±3.0
	Cut at 60 DAS	36.2±2.0	38.6±1.5	63.8±2.1	64.4±0.6
13-Nov-19	Cut at 40 DAS	31.0±1.4	26.4±0.5	52.1±0.5	51.1±0.8
	Cut at 50 DAS	32.0±0.6	31.1±0.4	56.3±0.3	55.6±0.7
	Cut at 60 DAS	35.0±1.7	34.6±0.8	59.6±0.8	60.5±0.7
26-Nov-19	Cut at 40 DAS	20.7±0.3	20.1±0.9	47.0±1.1	40.0±1.5
	Cut at 50 DAS	22.6±1.0	21.5±0.3	51.3±0.7	49.7±0.61
	Cut at 60 DAS	29.9±0.8	25.3±0.6	56.2±2.3	54.9±2.6
11-Dec-19	Cut at 40 DAS	18.8±0.4	18.2±0.2	44.4±1.3	45.2±1.5
	Cut at 50 DAS	20.1±0.7	19.5±0.3	49.6±0.6	47.5±1.7
	Cut at 60 DAS	27.3±1.5	25.6±1.6	53.3±0.6	51.7±1.2
26-Dec-19	Cut at 40 DAS	23.1±0.9	24.6±0.6	50.0±0.6	50.3±0.7
	Cut at 50 DAS	26.1±3.6	28.6±0.5	56.0±0.6	55.5±1.3
	Cut at 60 DAS	33.4±0.5	34.0±0.6	59.4±0.3	59.9±0.5
Mean					
Planting date (PD)	09-Oct-19	31.0^{b}		57.9^{a}	
	24-Oct-19	33.7^{a}		58.4^{a}	
	13-Nov-19	31.7^{b}		55.9^{b}	
	26-Nov-19	23.4^{d}		49.9^{c}	
	11-Dec-19	21.6^{e}		48.6^{c}	
	26-Dec-19	28.3^{c}		55.2^{b}	
Growth Stages (GS)	Cut at 40 DAS	24.7^{c}		49.7^{c}	
	Cut at 50 DAS	27.6^{b}		54.4^{b}	
	Cut at 60 DAS	32.5^{a}		58.8^{a}	
Genotypes (G)	OL-10	28.5^{a}		54.7^{a}	
	OL-11	28.0^{a}		53.9^{b}	
CD at 5%		PD = 1.62, GS = 0.933, G = NS, PD x GS = 2.82, PD x G = 2.64, GS x G = NS, PD x GS x G = NS		PD = 1.36, GS = 0.784, G = 0.533, PD x GS = 2.37, PD x G = 4.78, GS x G = NS, PD x GS x G = 2.47	

Values are mean ± standard deviation of three replications
NS = Non-significant
Values with same letter(s) are non-significant at 5% level of significance (Tukey's post-hoc test)

4.2.9 Dry matter content (%)

Dry matter is a good index of growth rate of the plant and the yield bearing capacity. Dry matter percentage is significantly (F=360.7, P<0.01)influenced by staggered planting dates and lied in the range from 9.7-13.0% (Table 13).The mean values showed that highest dry matter content was observed at Oct 24 planting date and lowest at Dec 11 planting date. Better environmental conditions were available during the initial planting dates as compared

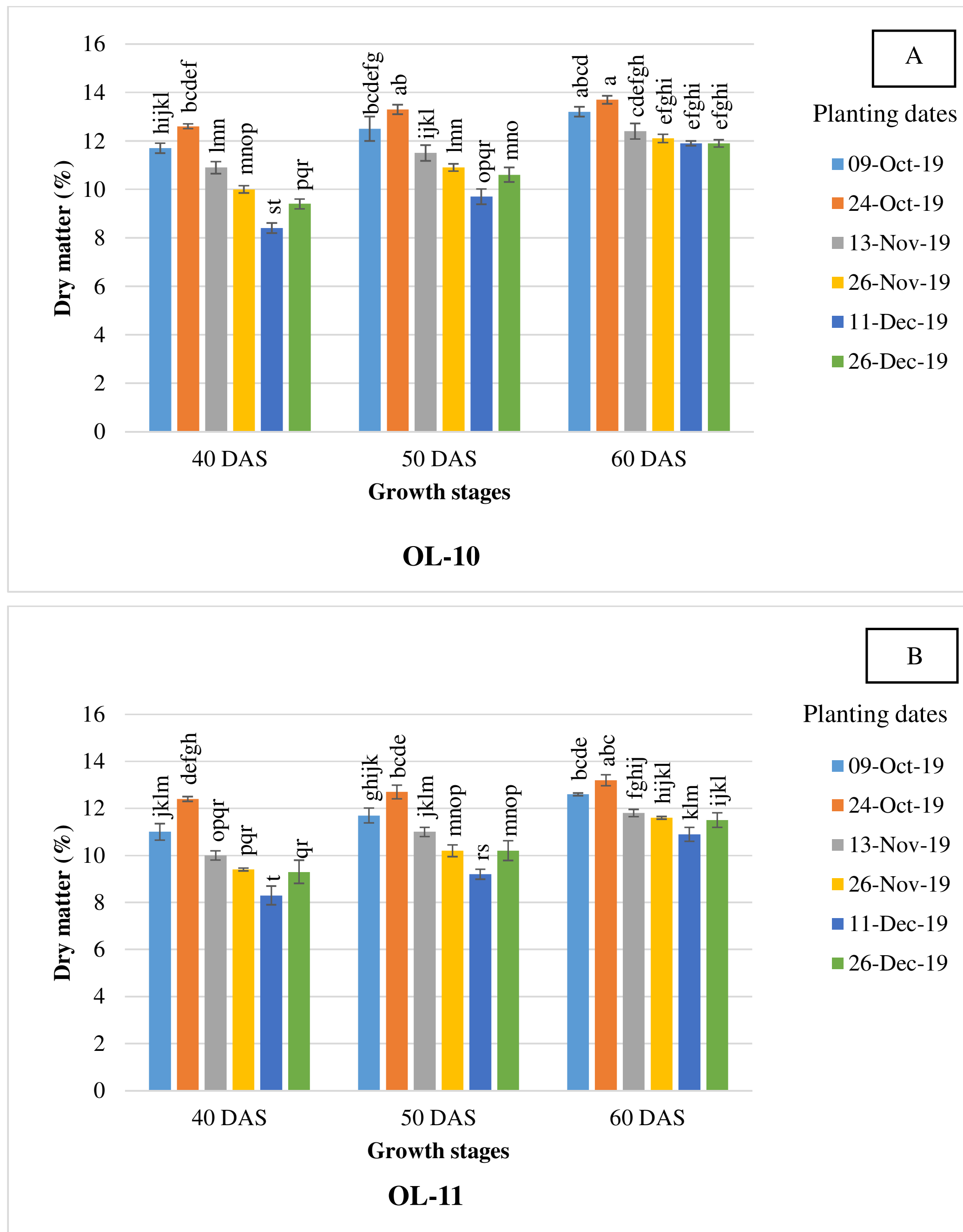


Fig 18 Dry matter content (%) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes (Error bars represents standard error)

to later ones which leads to more accumulation of dry matter. Similar significant variation in dry matter content was observed by Dar *et al* (2014), Singh *et al* (2016), Gupta *et al* (2010) and Salama (2019).

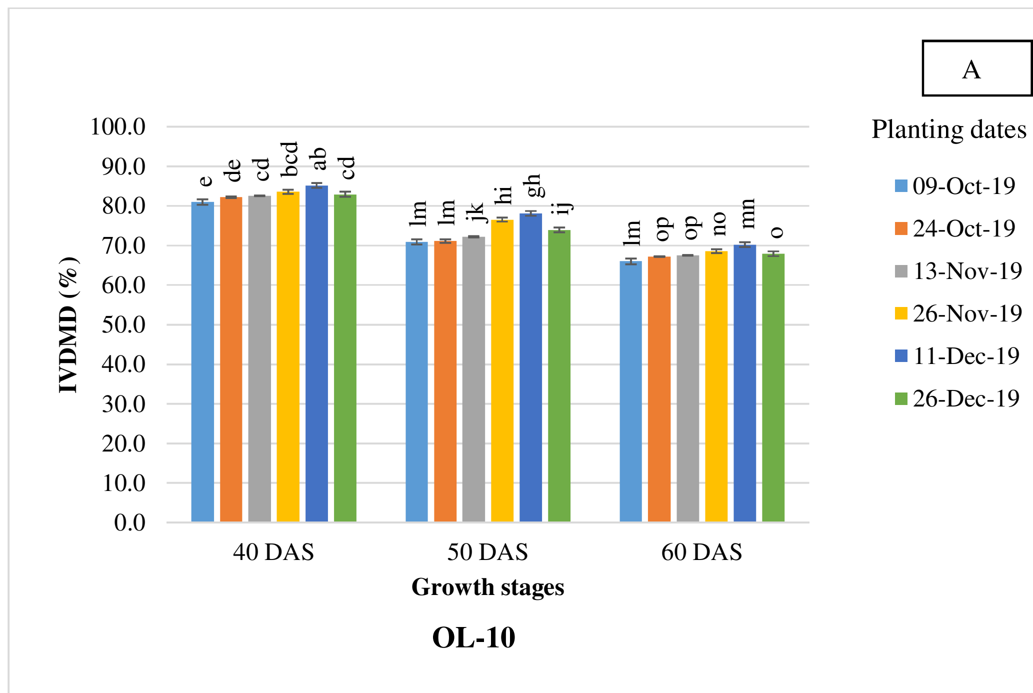
Significant (F=495.08, P<0.01) variation was observed for dry matter content among three growth stages that ranged from 10.3-12.2%. Highest dry matter content was found at 60 DAS and lowest at 40 DAS.Dry matter content increased with advancing maturity because of lignification of vegetative tissues (Lamaire and Belanger 2020). The variation in dry matter content among the growth stages were also reported by Talasila *et al* (2019) in fodder crop.

The dry matter content was significantly (F=108.53, P<0.01) high in OL-10 genotype (11.5%) as compared to OL-11 genotype (10.9%). Higher dry matter content in OL-10 might be due more plant height and tiller m^{-2}. Significant variation of dry matter content in different fodder genotypes were also observed by previous workers (Anjum *et al* 2014, Kaur *et al* 2018, Hameed *et al* 2014, Arif *et al* 2002, Mali and Choudhary 2011 and Kumar *et al* 2013). The three-way interaction of planting dates, growth stages and genotypes was found to be non-significant.

4.2.10 *In vitro* dry matter digestibility (IVDMD)

Digestibility is the main determinant of nutritive value of forage crops. Data for IVDMD content revealed significant variation among staggered planting dates (F=173.67, P<0.01) and lied in the range from 72.9-77.8% (Table 13). The highest IVDMD was exhibited at Dec 11 planting date and lowest was exhibited at Oct 9 planting date. Our results are in conformity with previous worker who reported more fibrous nature of phloem and xylem counterparts present in stem restricts the digestion of crop (Martinez *et al*2010). The variablility in IVDMD with planting time was also claimed by Salama (2019) in maize.

IVDMD decreased significantly (F=6580.3, P<0.01) with the advancement in age of the crop and lied in the range from 67.4-83.2%. Highest IVDMD was observed at 40 DAS followed by 50 DAS and 60 DAS. The higher IVDMD may be due to higher leaf stem ratio at earlier growth stages (Sindhu *et al* 2006). Since leaves contained lesser concentration of conducting vessels and other vascular tissues that are particularly recalcitrant to digestion (Ivan *et al* 2006). Similar values were reported by Kaur *et al* (2013) in barley and Malik *et al* (2015) in oats. The significantly (F=5.52, P<0.05) higher IVDMD in OL-11 genotype (75.2%) was because of more succulency and lesser plant height at almost all the planting dates and growth stages as compared to OL-10 genotype (74.9%).



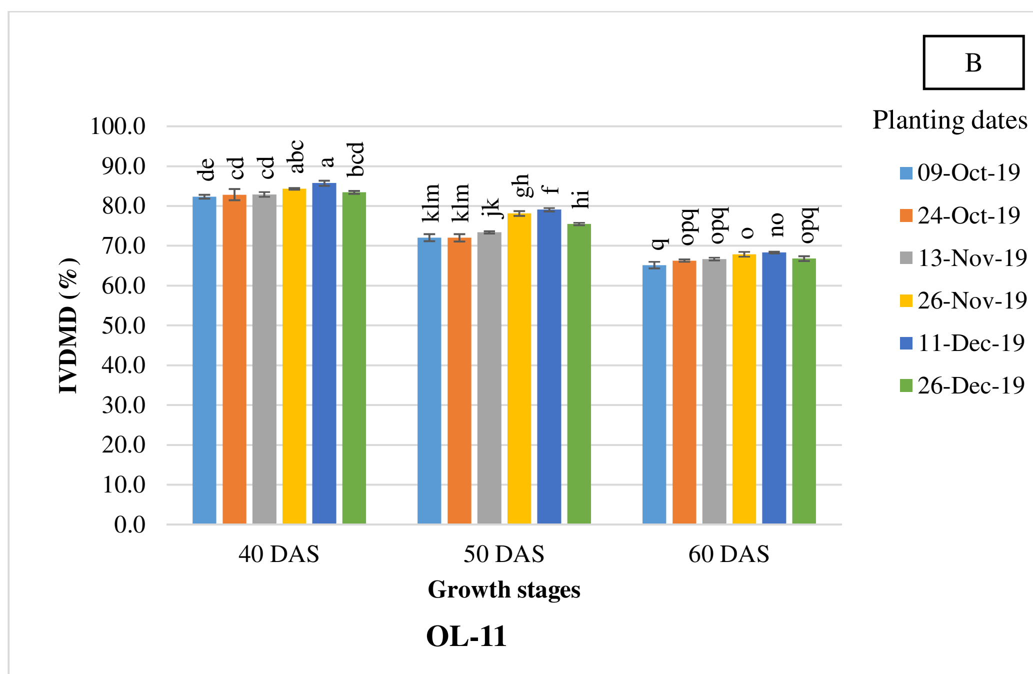


Fig 19 ***In vitro* dry matter digestibility (%) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes** (Error bars represents standard error)

Table 13 Dry matter and *In vitro* dry matter digestibility as influenced by planting dates and growth stages in oat genotypes (Percent, Dry matter basis).

PLANTING DATE	GROWTH STAGE (DAS)	Dry matter %		IVDMD (%)	
		Genotypes			
		OL-10	OL-11	OL-10	OL-11
09-Oct-19	Cut at 40 DAS	11.6±0.21	11.0±0.35	81.0±0.72	82.3±0.50
	Cut at 50 DAS	12.5±0.50	11.7±0.32	70.9±0.64	72.0±0.87
	Cut at 60 DAS	13.2±0.20	12.6±0.06	66.0±0.72	65.1±0.83
24-Oct-19	Cut at 40 DAS	12.6±0.10	12.4±0.10	82.2±0.20	82.8±1.40
	Cut at 50 DAS	13.3±0.20	12.7±0.29	71.1±0.46	72.0±0.92
	Cut at 60 DAS	13.7±0.17	13.2±0.23	67.5±0.12	66.3±0.31
13-Nov-19	Cut at 40 DAS	10.9±0.25	10.0±0.23	82.5±0.12	82.9±0.61
	Cut at 50 DAS	11.5±0.32	11.0±0.20	72.2±0.20	73.3±0.31
	Cut at 60 DAS	12.4±0.32	11.8±0.15	67.5±0.12	66.7±0.31
26-Nov-19	Cut at 40 DAS	10.0±0.15	9.4±0.06	83.6±0.51	84.3±0.26
	Cut at 50 DAS	10.9±0.15	10.2±0.25	76.5±0.50	78.1±0.61
	Cut at 60 DAS	12.1±0.17	11.6±0.06	68.6±0.51	67.9±0.61
11-Dec-19	Cut at 40 DAS	8.4±0.21	8.3±0.40	85.2±0.60	85.7±0.66
	Cut at 50 DAS	9.7±0.32	9.2±0.21	78.1±0.62	79.1±0.42
	Cut at 60 DAS	11.9±0.10	10.9±0.30	70.2±0.60	68.3±0.26
26-Dec-19	Cut at 40 DAS	9.4±0.20	9.3±0.49	82.9±0.64	83.4±0.40
	Cut at 50 DAS	10.6±0.30	10.2±0.42	73.9±0.64	75.5±0.31
	Cut at 60 DAS	11.9±0.15	11.5±0.31	67.9±0.64	66.8±0.59
Mean					
Planting date (PD)	09-Oct-19	12.1^{b}		72.9^{e}	
	24-Oct-19	13.0^{a}		73.7^{d}	
	13-Nov-19	11.3^{c}		74.2^{d}	
	26-Nov-19	10.7^{d}		76.5^{b}	
	11-Dec-19	9.7^{e}		77.8^{a}	
	26-Dec-19	10.5^{d}		75.1^{c}	
Growth Stages (GS)	Cut at 40 DAS	10.3^{c}		83.2^{a}	
	Cut at 50 DAS	11.1^{b}		74.4^{b}	
	Cut at 60 DAS	12.2^{a}		67.4^{c}	
Genotypes (G)	OL-10	11.5^{a}		74.9^{b}	
	OL-11	10.9^{b}		75.2^{a}	
CD at 5%		PD = 0.257, GS = 0.149, G = 0.101, PD x GS = 0.449, PD x G = NS, GS x G = NS, PD x GS x G = NS		PD = 0.572, GS = 0.331, G= 0.225, PD x GS = 1.00, PD x G = NS, GS x G = 0.572, PD x GS x G = NS	

Values are mean ± standard deviation of three replications
NS = Non-significant
Values with same letter(s) are non-significant at 5% level of significance (Tukey's post-hoc test)

4.2.11 Nitrate-N

Nitrate is the main form of nitrogen taken up by the plant roots from the soil thereby transported to the stem and leaf tissues. Normally, the nitrate is non-toxic but the elevated levels above 2000 ppm causes nitrate toxicity in plants (Sidhu *et al* 2011). The adverse climatic conditions such as cool weather, hail and frost causes damage to the plant by distrupting their photosynthetic machinery (Mishra 2011). This may results in accumulation of nitrate-N in excessive amount which ultimately leads to its toxicity in livestock (Uwah *et al* 2009). When this nitrate rich fodder is consumed by ruminants, the nitrate got converted into nitrite and the excess of it is absorbed by animal's blood stream. Due to this, the haemoglobin gets converted into met-haemoglobin which is a poor transporter of oxygen and as a result, the animal suffers difficulty in breathing (Benjamin 2006). Some animal death due to consumption of nitrate rich fodder are reported by several workers (Gontijo *et al* 2017 and Niyas *et al* 2019). The significant ($F=1285.6$, $P<0.01$) variation in nitrate content was observed with staggered planting and the effect was more predominant while shifting from optimum planting time to later ones. Among six planting dates, the nitrate-N ranged from 992-2746 ppm with highest nitrate-N content exhibited at Nov 26 planting date and lowest at Oct 9 planting date (Table 14). The uptake of nitrate may be more during later planting dates in comparison with its assimilation. The variability in weather conditions predominatly temperature and sunshine hours and also the NR activity leads to differential nitrate content with staggered planting. A previous worker observed the variation in nitrate-N content from 1056-1520 ppm with staggered planting in barley (Dhillon *et al* 2019).

Nitrate-N also varied significantly ($F=2497.9$, $P<0.01$) with the maturity of the plant and ranged between 1063-2407 ppm (Table 14). The nitrate-N was found to be highest at 40 DAS followed by 50 DAS and 60 DAS. A previous worker reported that nitrate-N content was more in mature plants as compared to younger ones (Anjana *et al* 2006). Earlier study reported that nitrate-N content in oat and sorghum decreased with increased dry matter content (Harada *et al* 2002). The present study is in conformity with earlier workers (Taute *et al* 2002, Sidhu *et al* 2011, Gairola *et al* 2009, Gulmezoglu *et al* 2010 and Singh *et al* 2000).

The significantly ($F=36.4$, $P<0.01$) higher nitrate-N was found in OL-10 genotype (1756 ppm) and lower in OL-11 genotype (1663 ppm). Though more efficient nitrogen metabolism was observed in OL-10 genotype, higher nitrate-N content may be due to higher uptake of N from the soil. The significant differences in nitrate-N among the different genotypes were also found by previous workers in different forage crops (Kaur and Goyal 2016 and Anjana *et al* 2007). The three-way interaction was significant ($F=5.37$, $P<0.01$) between planting dates, growth stages and genotypes.

4.2.12 Nitrite-N

Nitrite is formed from nitrate and its formation and assimilation is highly dependent

upon the activities of nitrate metabolizing enzymes (Pathak *et al* 2008). Generally, the concentration of nitrite found in plants was low because of its quick assimilation into ammonia by the enzyme nitrite reductase (Kuznetsova *et al* 2004). The assimilation of nitrite is very important because of its toxic nature. Significant variation was observed for nitrite-N content with staggered planting dates (F=1138.1, P<0.01). Among six planting dates, nitrite-N content lied in the range from 14.9-32.6 ppm (Table 14). Highest nitrite-N content was observed at Nov 26 planting date and lowest was observed at Oct 9 planting date. The activity

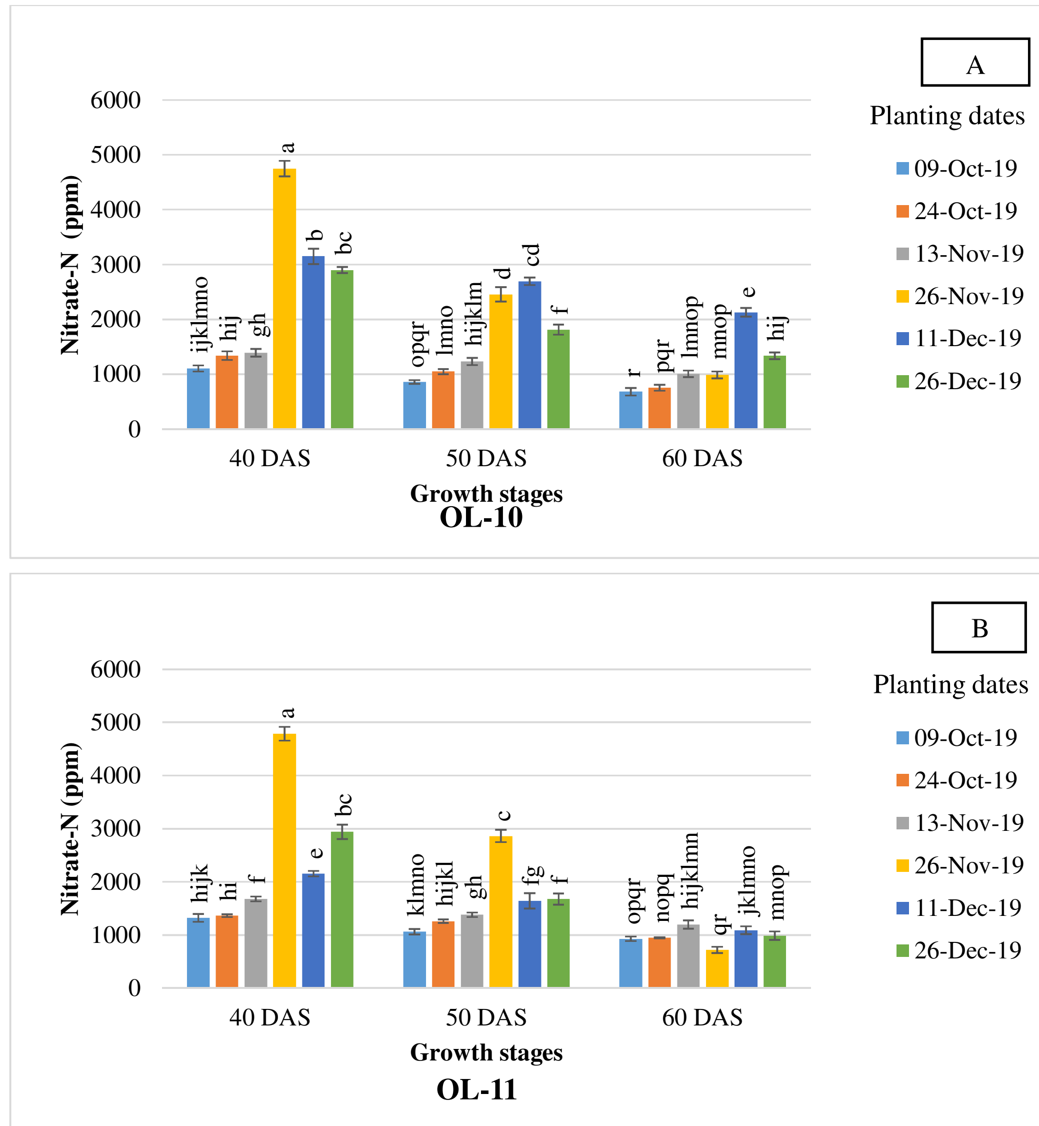


Fig 20 Nitrate-N content (ppm) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes (Error bars represents standard error)

of nitrite reductase was high during the initial three planting dates leading to low nitrite content, but during the later planting dates (Nov 26, Dec 11 and Dec 26), environmental stress resulted in the low NiR enzyme activity that leads to accumulation of nitrite content. Uwah *et al* (2009) also found higher amount of nitrite content in green leafy vegetables.

Nitrite content significantly decreased with the maturity of the plant (F=4100.5, P<0.01). Among three growth stages, nitrite-N content lied in the range from 12.9-29.8 ppm. Highest nitrite content was observed at 40 DAS and lowest at 60 DAS. Similar to nitrate, nitrite content was also observed low inmature plants. Nitrite content significantly (F=374.1, P<0.01) varied among two oat genotypes. Higher nitrite content was observed in OL-11 (22.7 ppm) genotype as compared to OL-10 (19.7 ppm) genotype. The assimilation of nitrite was rapid in OL-10 genotype because of high NiR activity which may be responsible for its lower nitrite content. Bahadoron *et al* (2016) also observed variable nitrite content in some vegetables and legumes.

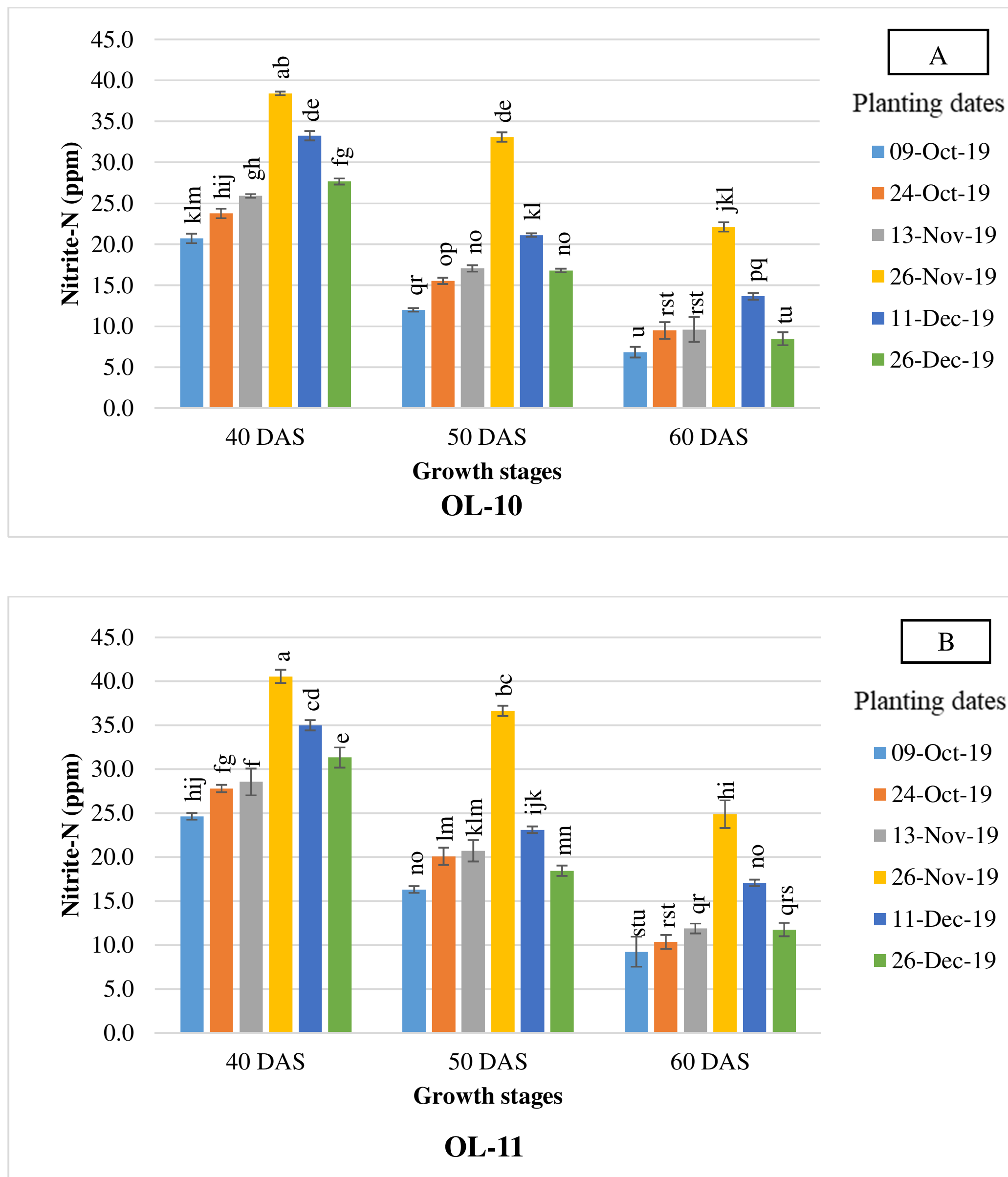


Fig 21 Nitrite-N content (ppm) as influenced by planting dates and growth stages in OL-10 (A) and OL-11 (B) genotypes (Error bars represents standard error)

Table 14 Nitrate-N and Nitrite-N as influenced by planting dates and growth stages in oat genotypes (ppm, Dry matter basis).

PLANTING DATES	GROWTH STAGES (DAS)	Nitrate-N (ppm)		Nitrite-N (ppm)	
		Genotypes			
		OL-10	OL-11	OL-10	OL-11
09-Oct-19	Cut at 40 DAS	1105±56.7	1322±71.6	20.7±0.58	24.6±0.38
	Cut at 50 DAS	858±34.4	1062±52.5	12.0±0.22	16.3±0.38
	Cut at 60 DAS	680±66.7	927±41.8	6.8±0.66	9.2±1.71
24-Oct-19	Cut at 40 DAS	1339±79.1	1365±26.0	23.8±0.58	27.8±0.44
	Cut at 50 DAS	1049±45.7	1261±34.4	15.5±0.38	20.1±1.00
	Cut at 60 DAS	754±56.7	949±13.0	9.5±1.00	10.4±0.79
13-Nov-19	Cut at 40 DAS	1391±72.4	1681±45.6	25.9±0.22	28.6±1.53
	Cut at 50 DAS	1231±66.7	1382±41.8	17.1±0.38	20.7±1.22
	Cut at 60 DAS	1005±61.4	1200±79.4	9.6±1.53	11.9±0.58
26-Nov-19	Cut at 40 DAS	4749±142.6	4788±130.9	38.4±0.22	40.6±0.76
	Cut at 50 DAS	2453±130.9	2778±117.2	33.1±0.58	36.6±0.58
	Cut at 60 DAS	988±65.0	719±58.6	22.1±0.58	24.9±1.58
11-Dec-19	Cut at 40 DAS	3150±139.0	2154±52.5	33.2±0.58	35.0±0.58
	Cut at 50 DAS	2691±68.8	1642±143.2	21.1±0.22	23.1±0.38
	Cut at 60 DAS	2128±78.4	1088±73.9	13.6±0.38	17.1±0.38
26-Dec-19	Cut at 40 DAS	2899±56.7	2942±137.8	27.7±0.38	31.3±1.16
	Cut at 50 DAS	1811±92.2	1677±103.2	16.8±0.22	18.4±0.58
	Cut at 60 DAS	1335±58.6	988±78.0	8.5±0.79	11.7±0.76
Mean					
Planting date (PD)	09-Oct-19	992^{f}		14.9^{e}	
	24-Oct-19	1119^{e}		17.8^{f}	
	13-Nov-19	1315^{d}		19.0^{c}	
	26-Nov-19	2746^{a}		32.6^{a}	
	11-Dec-19	2142^{b}		23.9^{b}	
	26-Dec-19	1942^{c}		19.1^{c}	
Growth Stages (GS)	Cut at 40 DAS	2407^{a}		29.8^{a}	
	Cut at 50 DAS	1658^{b}		20.9^{b}	
	Cut at 60 DAS	1063^{c}		12.9^{c}	
Genotypes (G)	OL-10	1756^{a}		19.7^{b}	
	OL-11	1663^{b}		22.7^{a}	
CD at 5%		PD = 80.0, GS = 45.6, G = 31.0, PD x GS = 138.0, PD x G = 128.8, GS x G =80.0, PD x GS x G = 143.9		PD = 1.09, GS = 0.891, G = 0.742, PD x GS = 1.39, PD x G = NS, GS x G =NS, PD x GS x G = 1.41	

Values are mean ± standard deviation of three replications
NS = Non-significant
Values with same letter(s) are non-significant at 5% level of significance (Tukey's post-hoc test)

4.3 Effect of different planting dates on yield and yield parameters in oat genotypes.

4.3.1 Leaf length

Statistical analysis showed significant effect of staggered planting dates (F=125.4, P<0.01) on leaf length. Among six planting dates, the leaf length ranged from 30.7-62.5 cm in OL-10 genotype and 30.0-57.5 cm in OL-11 genotype (Table 15). Highest leaf length was exhibited at Oct 24 planting date and lowest leaf length was observed at Dec 11 planting date. The variability in climate during initial and late planting dates may be responsible for differential leaf length during staggered planting time.

Table 15 Leaf length (cm), Leaf Breadth (cm) and Plant height (cm) as influenced by planting dates in oat genotypes at 60 DAS.

	Leaf Length (cm)		Leaf Breadth (cm)		Plant Height (cm)	
Planting Date	**OL-10**	**OL-11**	**OL-10**	**OL-11**	**OL-10**	**OL-11**
09-Oct-19	55.9±1.29[ab]	54.0±1.00[ab]	1.77±0.17[a]	1.80±0.05[a]	84.7±3.81[a]	66.5±6.24[b]
24-Oct-19	62.5±3.70[a]	57.5±0.76[ab]	1.75±0.30[ab]	1.59±0.20[abc]	85.1±7.08[a]	67.4±3.06[b]
13-Nov-19	56.5±2.43[ab]	52.1±1.81[b]	1.77±0.06[a]	1.93±0.06[a]	79.2±1.91[a]	64.7±1.06[bc]
26-Nov-19	35.6±6.40[c]	27.7±2.16[c]	1.41±0.04[bc]	1.41±0.05[bc]	52.3±4.52[de]	43.7±3.33[efg]
11-Dec-19	30.7±3.72[c]	30.0±3.58[c]	1.36±0.07[c]	1.33±0.01[c]	41.1±0.82[fg]	34.3±2.26[g]
26-Dec-19	35.0±0.20[c]	30.5±0.95[c]	1.38±0.00[c]	1.31±0.04[c]	54.9±2.03[cd]	46.5±1.02[def]
CD at 5%	Planting dates (PD) = 5.41, Genotypes (G) = 2.08, PD x G = NS		Planting dates (PD) = 0.213, Genotypes (G) = NS, PD x G = NS		Planting dates (PD) = 5.22, Genotypes (G) = 2.46, PD x G = 6.10	

Values are mean ± standard deviation of three replications

NS = Non-significant

Values with same letter(s) are non-significant at 5% level of significance (Tukey's post-hoc test)

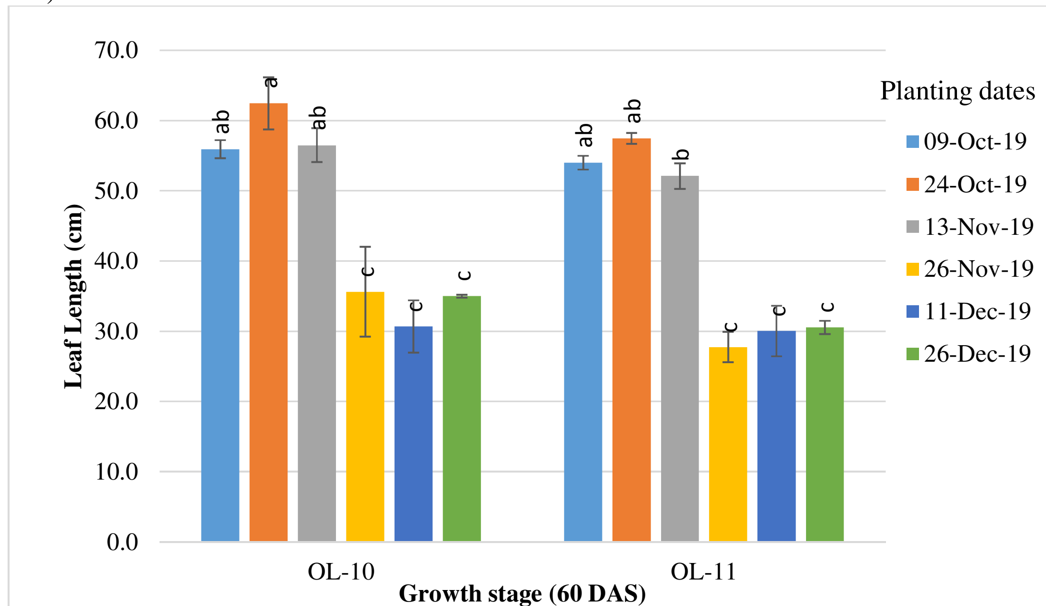


Fig 22 Leaf length (cm) as influenced by planting dates in oat genotypes (Error bars represents standard error)

4.3.2 Leaf breadth

Significant effect of staggered planting dates (F=22.4, P<0.01) on leaf breadth was observed. Among six planting dates, the leaf breadth ranged from 1.36-1.77 cm in OL-10 genotype and 1.31-1.80 cm in OL-11 genotype (Table 15). Highest leaf breadth was exhibited at Nov 13 planting date and lowest leaf breadth was exhibited at Dec 11 planting date.

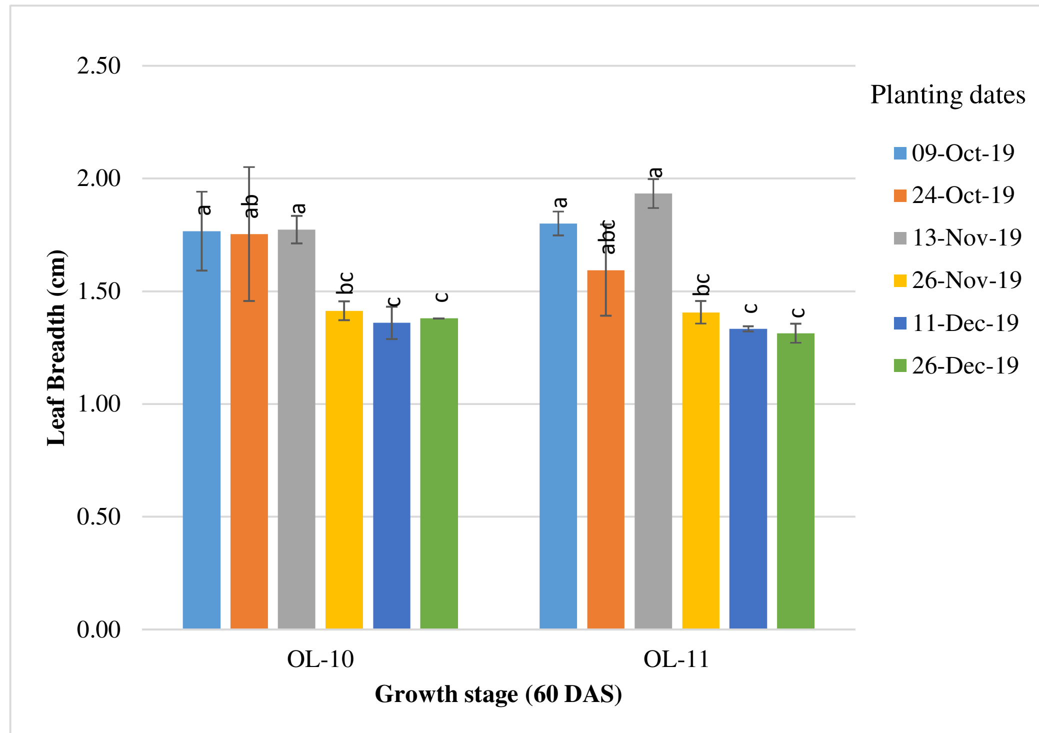


Fig 23 Leaf Breadth (cm) as influenced by planting dates in oat genotypes (Error bars represents standard error)

4.3.3 Plant height

Significant effect of staggered planting (F=130.8, P<0.01) was observed on the plant height. Among six planting dates, the plant height varied from 41.1-85.1 cm in OL-10 and 34.3-67.4 cm in OL-11 genotype (Table 15). Highest plant height was recorded at Oct 24 planting date and lowest plant height was recorded at Dec 11 planting date. Since plant growth is mainly governed by auxin biosynthesis which is directly proportional to temperature. Low temperature during the late planting time slowed down the auxin synthesis and may leads to less plant height. Similar findings were reported by Kalwar *et al* (2018), Mumtaz *et al* (2015) in wheat, Dhillon *et al* (2019) in barley and Sharma *et al* (2017) in oats.

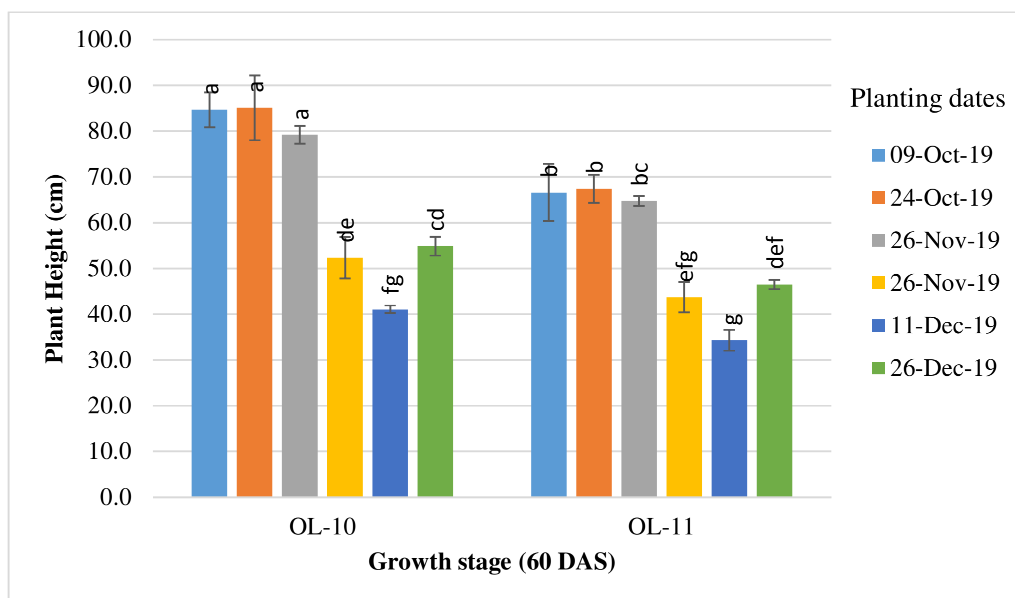


Fig 24 Plant Height (cm) as influenced by planting dates in oat genotypes (Error bars represents standard error)

4.3.4 Tillers sq m^{-1}

Significant difference of staggered planting dates (F=12.2, p<0.01) on number of tillers was observed. Among six planting dates, the number of tillers varied from 363-619 in OL-10 genotype and 336-565 in OL-11 genotype (Table 16). Maximum tillers square m^{-1} were recorded at Oct 24 planting date while minimum were recorded at Nov 26 planting date. The delay in planting time significantly reduced the tillers which may be attributed to delayed germination and slow growth rate because of adverse environmental conditions. The present investigation is in agreement with Buttar *et al* (2018).

Table 16 Tillers sq m^{-1}and number of leaves per plant as influenced by planting dates in oat genotypes.

	Tillers sq m^{-1}		**Number of leaves per plant**	
Planting Date	**OL-10**	**OL-11**	**OL-10**	**OL-11**
09-Oct-19	474.7±94.8^{abc}	469.3±48.2^{abc}	6.7±0.58^{a}	6.3±0.58^{ab}
24-Oct-19	618.7±64.7^{a}	565.3±84.8^{ab}	5.3±0.58^{bc}	5.0±0.00^{cd}
13-Nov-19	514.7±46.9^{abc}	480.0±34.9^{abc}	5.3±0.58^{bc}	4.7±0.58^{cd}
26-Nov-19	362.7±12.2^{c}	336±28.8^{c}	4.0±0.00^{d}	4.0±0.00^{d}
11-Dec-19	386.7±50.8^{bc}	384±56.0^{bc}	4.0±0.00^{d}	4.0±0.00^{d}
26-Dec-19	442.7±40.3^{abc}	405.3±108.9^{bc}	4.3±0.58^{cd}	4.0±0.00^{d}
CD at 5%	Planting dates (PD) = 109.9, Genotypes (G) = NS, PD x G = NS		Planting dates (PD) = 0.745, Genotypes (G) = NS, PD x G = NS	

Values are mean ± standard deviation of three replications
NS = Non-significant
Values with same letter(s) are non-significant at 5% level of significance (Tukey's post-hoc test)

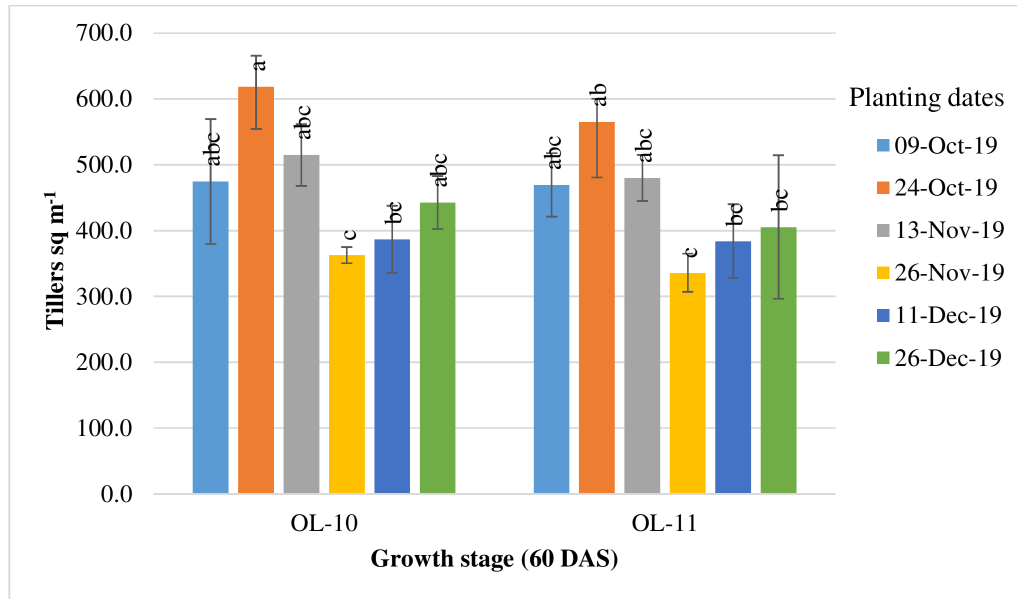


Fig 25 Tillers sq m^{-1} as influenced by planting dates in oat genotypes (Error bars represents standard error)

4.3.5 Number of leaves per plant

The effect of staggered planting dates (F=33.2, P<0.01) on number of leaves per plant was significant. Among six planting dates, the number of leaves per plant varied from 4-6.7 in OL-10 genotype and 4-6.3 in OL-11 genotype (Table 16). Highest number of leaves were recorded at Oct 9 planting date and lowest was recorded at Nov 26 and Dec 11 planting dates which was at par with Dec 26 planting date. Sharma *et al* (2017) observed that number of leaves per plant in oat lied in the range from 7.2-13.2 with staggered planting time.

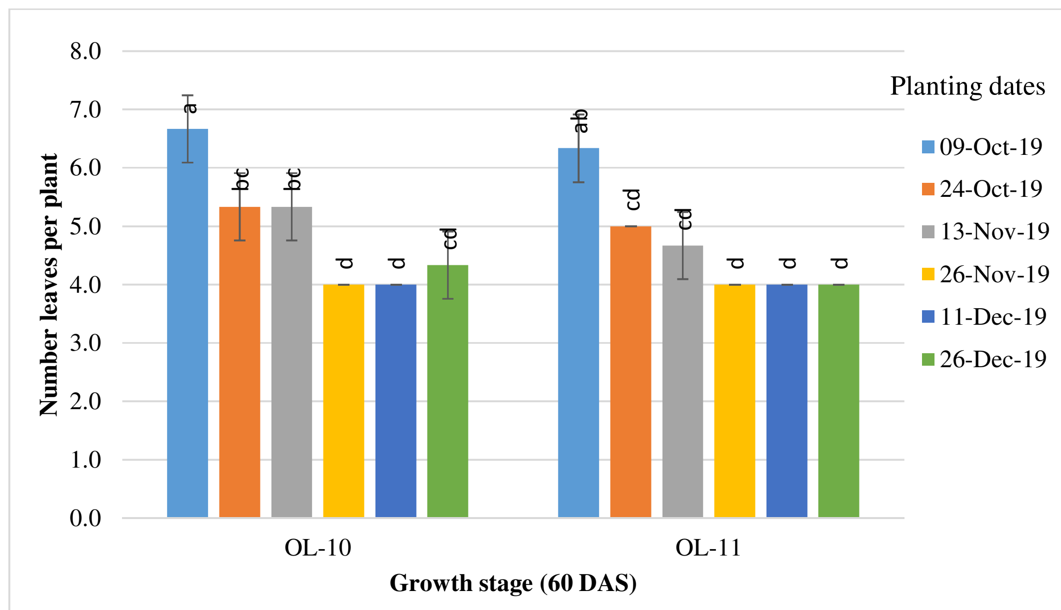


Fig 26 Number of leaves per plantas influenced by planting dates in oat genotypes (Error bars represents standard error)

4.3.6 Green fodder yield

Green fodder yield is an important component in determining the efficiency of forage crop. Table 19 revealed that staggered planting dates (F=75.9, P<0.01) had a significant effect on green fodder yield. The mean values showed that among six planting dates, green fodder yield lied in the range from 75.7-180 q/ha in OL-10 genotype and 71.3-150 q/ha in OL-11 genotype (Table 17). The variability in green fodder yield in both the genotypes was because of differential growth potential, OL-10 being fast growing with high regeneration potential and OL-11, the relatively slow growing single cut variety. Genotypic differences were also reported by Naeem *et al* (2006) and Lodhi *et al* (2009) in oat. Highest green fodder yield was observed at Oct 24 planting date and lowest green fodder yield was observed at Dec 11 planting date. All the metabolic processes such as photosynthesis, photoperiodism, respiration and transpiration were uplifted by favourable climatic conditions that resulted into rapid growth of plants (Jehangir *et al* 2013). In addition, better vegetative growth during optimum (Oct 24) and sub-optimum planting time (Oct 9 and Nov 13) was also because of tillers sq metre^{-1}, plant height, leaf length and breadth over delayed planting time. Similar results were reported by Sharma *et al* (2017) in oat.

Table 17 Green fodder yield (q/ha), Dry matter yield (q/ha) and crude protein yield (q/ha) as influenced by planting dates in oat genotypes.

	Green fodder yield (q/ha)		**Dry matter yield (q/ha)**		**Crude protein yield (q/ha)**	
Planting Date	**OL-10**	**OL-11**	**OL-10**	**OL-11**	**OL-10**	**OL-11**
09-Oct-19	165.3±28.4^{ab}	140.0±9.2^{b}	21.8±3.52^{ab}	17.7±1.24^{bc}	2.17±0.45^{bc}	1.40±0.03^{def}
24-Oct-19	180.0±20.0^{a}	150.0±5.6^{ab}	24.7±2.72^{a}	19.8±0.51^{bc}	2.93±0.35^{a}	1.97±0.05^{cd}
13-Nov-19	162.7±6.8^{ab}	145.0±4.6^{ab}	20.1±0.78^{bc}	17.1±0.34^{c}	2.67±0.15^{ab}	1.87±0.06^{cde}
26-Nov-19	82.0±8.7^{c}	73.0±7.2^{c}	9.9±1.15^{d}	8.4±0.80^{d}	1.47±0.15^{def}	1.07±0.14^{f}
11-Dec-19	75.7±5.5^{c}	71.3±1.5^{c}	9.0±0.70^{d}	7.8±0.35^{d}	1.47±0.15^{def}	1.30±0.08^{ef}
26-Dec-19	98.0±10.6^{c}	82.0±2.6^{c}	11.6±1.14^{d}	9.4±0.50^{d}	1.50±0.10^{def}	1.13±0.04^{f}
CD at 5%	Planting dates (PD) = 21.6, Genotypes (G) = 8.29, PD x G = NS		CD at 5%: Planting date (PD) = 2.73, Genotypes (G) = 1.05, PD x G = NS		Planting dates (PD) = 0.290, Genotypes (G) = 0.137,PD x G = 0.339	

Values are mean ± standard deviation of three replications

NS = Non-significant

Values with same letter(s) are non-significant at 5% level of significance (Tukey's post-hoc test)

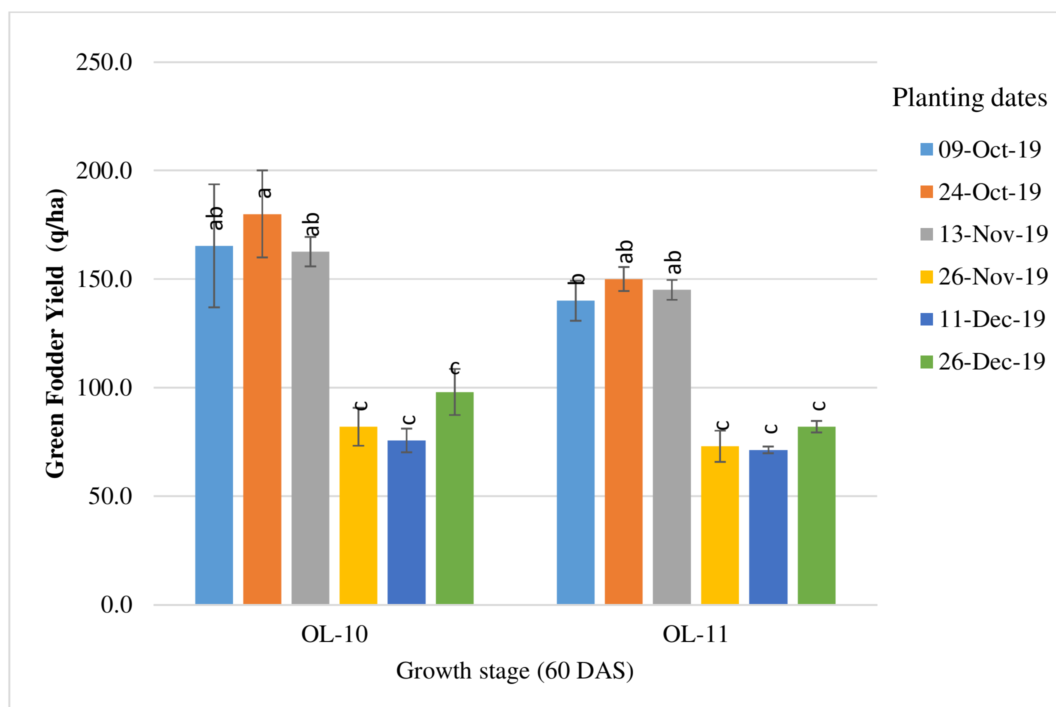


Fig 27 Green fodder yield (q/ha) as influenced by planting dates in oat genotypes (Error bars represents standard error)

4.3.7 Dry matter yield

Dry matter yield is an important parameter of fodder crops because the production of nutrients per unit area as well as the quality of the crop is estimated from dry matter. Dry matter yield depends upon the green fodder yield and moisture content at the time of cutting. Significant variation (F=96.5, P<0.01) was observed for dry matter yield among six planting dates. The dry matter yield lied in the range from 9.0-24.7 q/ha in OL-10 genotype and 7.8-19.8 q/ha in OL-11 genotype (Table 17). Highest dry matter yield was exhibited at Oct 24 planting date and lowest dry matter yield was exhibited at Dec 11 planting date. The present investigation is in conformity with the previous findings of Dar *et al* (2014) in oats. The more dry matter yield during the initial planting dates (i.e. Oct 9, Oct 24 and Nov 13) was due to more green fodder yield at that planting time. Moreover, photosynthetic activity of the leaves was also high because of more sunshine hours available during the initial planting time which is essential for the accumulation of dry matter (Sharma *et al* 2017). During the late planting dates (i.e. Nov 26, Dec 11 and Dec 26), the available nutrients mainly nitrogen was not utilized properly which decreased cell division and elongation process resulting in decreased dry matter yield (Kumari *et al* 2014).

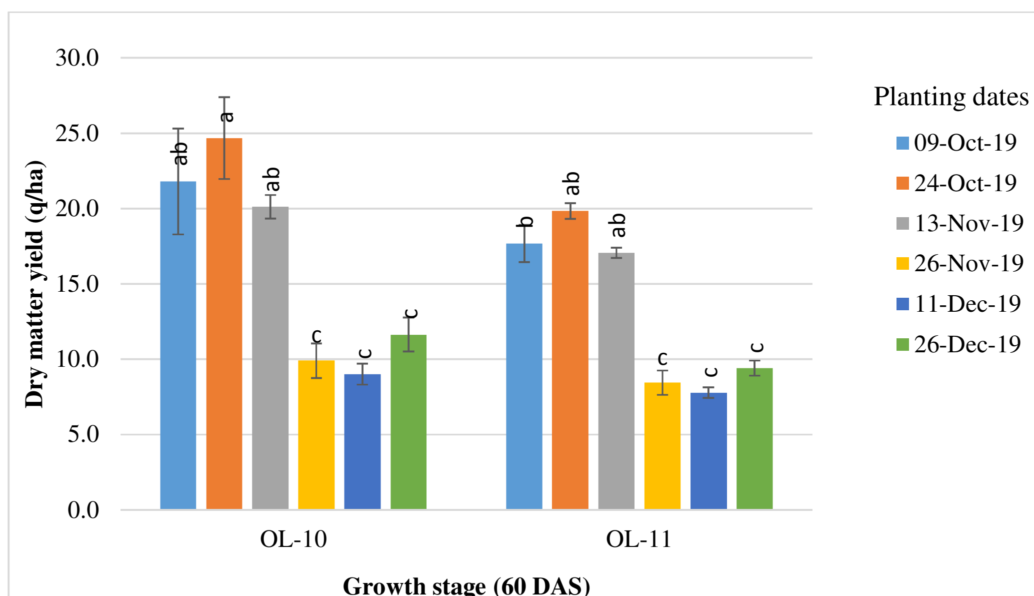


Fig 28 Dry matter yield (q/ha) as influenced by planting dates in oat genotypes (Error bars represents standard error)

4.3.8 Crude protein yield

The data presented in table revealed significant effect (F=40.4, P<0.01) of staggered planting dates on crude protein yield. The data revealed that crude protein content ranged from 1.47-2.93 q/ha in OL-10 genotype and from 1.07-1.97 q/ha in OL-11 genotype (Table 17). Maximum crude protein yield was exhibited at Oct 24 planting date and lowest crude protein yield was exhibited at Nov 26 planting date. Shekara *et al* (2012) reported that crude protein yield varied from 4.4-6.4 q/ha in different oat genotypes.

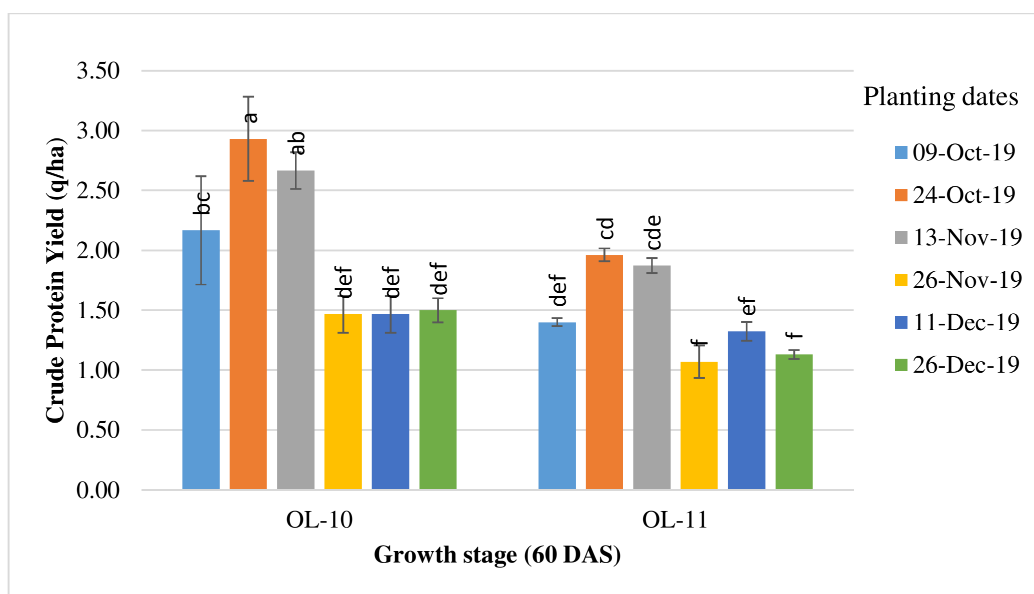


Fig 29 Crude protein yield (q/ha) as influenced by planting date sin oat genotypes (Error bars represents standard error)

Table 18 Correlation analysis of nitrogen metabolic enzymes, nitrate-N, minimum temperature and sunshine hours with staggered planting dates in oat genotypes.

Parameters	Planting dates	NR	NiR	GS	GOGAT	GDH	GPT	GOT	Nitrate-N	Min Temp	Sun hrs.	Planting dates	Parameters
NR	Oct-09	1	.822*	.748	.803	-.180	.729	.993**	-.514	-.558	0.067	Nov-26	NR
	Oct-24	1	.888*	.852*	.889*	.898*	.103	.963**	-.133	-.412	.719	Dec-11	
	Nov-13	1	.567	.196	.620	.236	.334	.257	-.476	.280	.809	Dec-26	
NiR	Oct-09	.819**	1	.907*	.482	.147	.908	.757	-.455	-.464	.180	Nov-26	NiR
	Oct-24	.809	1	.525	.762	.647	-.136	.830*	.123	-.554	.656	Dec-11	
	Nov-13	.567	1	-.309	.082	-.214	.527	.278	.090	-.322	.520	Dec-26	
GS	Oct-09	-.492	-.865**	1	.618	.005	.723	.708	-771	-.768	.559	Nov-26	GS
	Oct-24	.668	.640	1	.844*	.971**	.213	.870*	-.491	-.247	.685	Dec-11	
	Nov-13	.315	.866*	1	.851*	.847*	-.745	-.694	-.827*	.968	-.048	Dec-26	
GOGAT	Oct-09	-.529*	-.906**	.965**	1	-.642	.237	.839*	-.779	-.825	.398	Nov-26	GOGAT
	Oct-24	.428	.169	.847*	1	.943**	-.181	.966**	-.445	-.705	.943**	Dec-11	
	Nov 13	.205	.691	.910	1	.772	-.396	-.414	-.908*	.898	.413	Dec-26	
GDH	Oct-09	-.507	-.855**	.826**	.899**	1	.374	-.228	.404	.437	-.159	Nov-26	GDH
	Oct-24	.597	.433	.902*	.921**	1	.017	.936**	-.524	-.466	0.835*	Dec-11	
	Nov-13	-.053	.505	.844*	.958**	1	-.357	-.281	-.941**	.898	.280	Dec-26	
GPT	Oct-09	-.230	.627*	.703**	.801**	.865**	1	.670	-.192	-.188	-.121	Nov-26	GPT
	Oct-24	.950**	.887*	.816*	.527	.716	1	.035	.428	.706	-.462	Dec-11	
	Nov-13	-.213	-.412	-.185	.164	.287	1	.960**	.271	-.613	.669	Dec-26	
GOT	Oct-09	-.382	-.555*	.518*	.493	.717**	.369*	1	-.539	-.586	.078	Nov-26	GOT
	Oct-24	.932**	.912*	.822*	.509	.243	.989**	1	-.248	-.553	.831*	Dec-11	
	Nov-13	-.528	-.623	-.293	.086	.243	.857*	1	.216	-.537	.686	Dec-26	
Nitrate-N	Oct-09	.684**	.949**	-.887**	-.929**	-.903**	-.709**	-.613*	1	.996	-.833*	Nov-26	Nitrate-N
	Oct-24	.344	.771	.647	.199	.342	.568	.604	1	.412	-.598	Dec-11	
	Nov-26	-.126	.532	.634	.409	.393	-.712	-.540	1	-.922	-.481	Dec-26	
Min Temp	Oct-09	.763**	.971**	-.854**	-.898**	-.848**	-.569*	-.534*	.963**	1	-.812*	Nov-26	Min temp
	Oct-24	.113	.045	.778	.906*	.768	.296	.289	.356	1	-.872*	Dec-11	
	Nov-13	.836*	.467	.004	-.205	-.484	-.564	-.742	-.040	1	.136	Dec-26	
Sun hrs.	Oct-09	.551*	.807**	-.803**	-.848**	-.815**	-.860**	-.662**	.853**	.730**	1	Nov-26	Sun hrs.
	Oct-24	.648	.967**	.580	.076	.340	.784	.810	.871*	.047	1	Dec-11	
	Nov-13	-.654	-.941**	-.750	-.468	-.281	.682	.815*	-.648	-.609	1	Dec-26	

* and ** are significant at 0.05 and 0.01 levels of significance

Left side of the diagonal represents optimum (Oct 24) and sub-optimum (Oct 9 and Nov 13) planting dates and right side of the diagonal represents late (Nov 26, Dec 11 and Dec 26) planting dates.

Correlation study

Correlation analysis depicted relationship between nitrogen metabolic enzymes, nitrate-N content and weather conditions (minimum temperature and sunsine hours). NR showed positive correlation with minimum temperature at Oct 9 (r = 0.763, P<0.01) and Nov 13 (r = 0.836, P<0.05) and also with sunshine hours at Oct 9 (r = 0.551, P<0.01) planting date. NiR depicted positive coorelation with minimum temperature at Oct 9 (r = 0.971, P<0.01) and with sunshine hours at Oct 9 (r = 0.807, P<0.01) and Oct 24 (r = 0.967, P<0.01). The GS (r = -0.854, P<0.01), GOGAT (r = -0898, P<0.01), GDH(r = -0.848, P<0.01), GPT (r = -0.569, P<0.05) and GOT (r = -0.534, P<0.05) depicted negative correlation with minimum temperature at Oct 9 planting date (Table 18). The GS also showed positive correlation with GOGAT, GDH, GPT and GOT at almost all planting dates.

Overall correlation analysis of nutritional composition and digestibility depicted that DM content was negatively correlated with CP (r = -0.759, P<0.01), NPCP (r = -0.584, P<0.01), TP (r = -0.762, P<0.01) and IVDMD (r = -0.702, P<0.01) but positively correlated withADF (r = 0.836, P<0.01) and NDF (r = 0.820, P<0.01) content. The CP content was positively correlated with NPCP (r = 0.866, P<0.01), TP (r = 0.928, P<0.01) and ash (r = 0.204, P<0.05) and IVDMD (r = 0.876, P<0.01) and negatively correlated with ADF (r = 0.677, P<0.01) and NDF (r = -0.846, P<0.01) content (Table 19).

Table 19 Overall correlation analysis of nutritional composition and digestibility in oat genotypes

	DM	CP	NPCP	TP	Ash	CF	ADF	NDF	IVDMD
DM	1								
CP	-.759**	1							
NPCP	-.584**	.866**	1						
TP	-.762**	.928**	.619**	1					
Ash	.177	.204*	.301**	.091	1				
CF	.410**	-.369**	-.223*	-.426**	.610**	1			
ADF	.836**	-.746**	-.672**	-.677**	.121	.365**	1		
NDF	.820**	-.846**	-.725**	-.789**	-.011	.353**	.874**	1	
IVDMD	-.702**	.876**	.766**	.805**	.471**	-.019	-.703**	-.796**	1

* and ** are significant at 0.05 and 0.01 levels of significance

CHAPTER-V

SUMMARY

Oat is a major cereal forage crop cultivated in the northern, central and eastern regions of India. It is sown in the winter season and considered to be one of the best dual purpose crops that fit well into the platter of human and cattle as well. Though India is among the leading producers of milk and milk products, our country faces net deficit of 64% feeds, 21.9% dry crop residues and 61.1% green fodder. In order to maintain the health of livestock, there is need for good quality green fodder in sufficient quantities. Planting date is one of the major factor to determine the ability of the crop to withstand different environment conditions. Temperature and sunshine hours during crop developmental process are the major factors affecting forage quality and yield of oat. Nitrogen metabolic enzymes are also affected by many factors like sunlight, frost, hail etc. Nitrate reductase activity is directly related to nitrate-N content in crops. A sudden decline in plant growth under any stressful condition causes accumulation of nitrates in the plant. Normally, these plants are consumed by ruminant animals, the nitrate breaks down to ammonia and then gets converted into microbial protein by the bacteria present in the rumen. But when ingested in higher amounts, accumulation of nitrite in the rumen may occur, which is an intermediate of nitrate to ammonia conversion. Due to this, release of oxygen to various body tissues become difficult resulting in tissue asphyxiation and death of the animal.

The present work was aimed at studying the effect of different planting dates and growth stages on nitrogen metabolic enzymes, nutritional composition and digestibility as well as on yield and yield parameters in oat genotypes. The activities of NR and NiR were observed high during optimum (Oct 24) and sub-optimum (Oct 9 and Nov 13) planting dates but were low during late planting time (Nov 26, Dec 11 and Dec 26). The activities of GS, GOGAT, GDH, GPT and GOT were observed higher during late planting dates but were stable or slightly low during optimum and sub-optimum planting dates. Highest NR and NiR activity was observed at Oct 9 planting date and among three growth stages, the activity varied according to sunshine hours. Highest GS and GOGAT was observed at Dec 11 and Nov 26 planting date and among three growth stages, the trend of both the enzymes was similar and increased from 40 to 60 DAS at some planting dates (Nov 26, Dec 11 and Dec 26). GDH activity was observed highest at Nov 26 planting date and among three growth stages it increased with maturity from 40 to 60 DAS at some planting dates (Dec 11 and Dec 26). GPT and GOT activity was highest at Nov 13 and Dec 11 planting date. OL-10 genotype exhibited higher activities of all nitrogen metabolic enzymes in comparison with OL-11 genotype.

Chlorophyll content was highest at Oct 9 planting date and among growth stages, it

varied according to temperature and sunshine hours. Delay in planting time resulted in increased free amino acids content, nitrate content and nitrite content. Highest free amino acid content was observed at Dec 26 planting date while highest nitrate and nitrite content was observed at Nov 26 planting date. Among three growth stages, free amino acids content increased from 40-60 DAS while nitrate and nitrite content decreased from 40 to 60 DAS. The chlorophyll content and nitrate content was observed higher in OL-10 genotype while nitrite content was higher in OL-11 genotype.

The optimum and sub-optimum planting dates were characterized by higher dry matter, ADF, NDF, ash, crude fat content but low crude protein (CP), non-protein crude protein (NPCP), true protein and IVDMD. Highest dry matter, ADF, NDF and ash were observed at Oct 24 planting date and highest crude fat content was observed at Oct 9 planting date. Late planting dates were characterized by higher CP, NPCP, true protein and IVDMD and all these parameters were observed highest at Dec 11 planting date. Among three growth stages, dry matter, ADF and NDF content increased from 40 to 60 DAS while CP, true protein, ash and IVDMD decreased with the advancing maturity of vegetative fractions of plants. All the quality traits except IVDMD content were observed higher in OL-10 genotype in comparison with OL-11 genotype.

Yield and yield parameters also varied significantly with staggered planting dates. Leaf length, leaf breadth, plant height, tillers sq m^{-1} and number of leaves per plant were observed high during optimum and sub-optimum planting dates. Green fodder yield, dry matter yield and crude protein yield also decreased with delay in planting time. Yield and yield attributes such as plant height were observed higher in OL-10 genotype in comparison with OL-11 genotype.

Hence, staggered planting proves to be one of the most influential factor affecting nitrogen metabolic enzymes, oat quality traits, digestibility and yield. Additionally, maturity of vegetative fractions of plants also influences nitrogen metabolic enzymes, nutritional composition, digestibility and antinutritional composition. Late planting leads to the reduction in the fodder yield, hence optimum time of planting should be recommended to the farmers to avoid temperature and light stress during vegetative growth of the oat crop. Overall, OL-10 responded better in terms of nitrogen metabolic enzyme activities, quality traits as well as yield of the crop in comparison with OL-11 genotype.

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