

**CONSTRUCTION OF FRAMEWORK LINKAGE MAP AND
GENETIC DISSECTION OF BIOMASS RELATED TRAITS
IN BAJRA-NAPIER HYBRID**

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Thesis

Master of Science in Agriculture
(Genetics and Plant Breeding)



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CONSTRUCTION OF FRAMEWORK LINKAGE MAP AND GENETIC DISSECTION OF BIOMASS RELATED TRAITS IN BAJRA-NAPIER HYBRID

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By

Alapati Revanth Satya Sai Hanuman Gupta

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CERTIFICATE-I

Certified that **Mr. Alapati Revanth Satya Sai Hanuman Gupta. ID. No. RLBCAU/AG/PG/0051/21** has satisfactorily pursued his course of research for not less than IV semesters and that the thesis entitled **“Construction of framework linkage map and genetic dissection of biomass related traits in Bajra-Napier hybrid”** submitted by him to the Rani Lakshmi Bai Central Agricultural University, Jhansi 284003 (U.P.) in partial fulfilment of the requirements for the award of the degree of Master of Science in Agriculture in the subject of Genetics and Plant Breeding is the result of original research work conducted by him under my supervision and is sufficiently of a high standard to warrant its presentation to the examination.

I also certify that the thesis or part thereof has not been previously submitted by him for a degree/diploma of any University.

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CERTIFICATE-II

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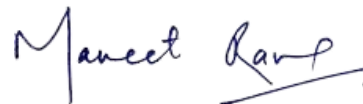
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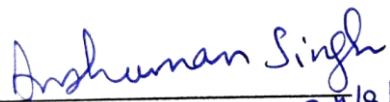


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To,
The Director Education,
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**Subject: Submission of M.Sc. (Ag.) in Genetics and Plant Breeding dissertation
report of Mr. Alapati Revanth Satya Sai Hanuman Gupta.**

Sir,

I enclose herewith a thesis entitled “**Construction of framework linkage map and genetic dissection of biomass related traits in Bajra-Napier Hybrid**” is an original piece of work carried out by Mr Alapati Revanth Satya Sai Hanuman Gupta, RLBCAU/AG/PG/051/21 in partial fulfilment for the award of degree of Master of Science in Agriculture (Genetic and Plant Breeding), College of Agriculture, RLBCAU, Jhansi (U.P).

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Thanking You.


(Amaresh Chandra)

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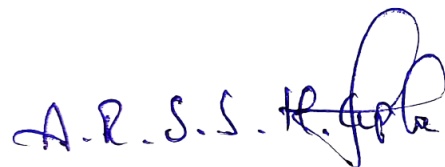
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Needless to say, all omissions and errors are mine.

Place: RLBCAU, Jhansi

Dated: August, 2023



Alapati Revanth Satya Sai Hanuman Gupta

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LIST OF ABBREVIATIONS USED

Abbreviation	Meaning
%	Per cent
*	Significant at 5% level
**	Significant at 1% level
***	Significant at 0.1% level
µg	Microgram
µl	Microlitre
µM	Micro molar
AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis of Variance
BILs	Backcross Inbred lines
BN	Bajra Napier
bp	base pair
BxN	Bajra crossed with Napier
CHL	Chlorophyll
CIM	Composite Interval Mapping
CISPs	Conserved-Intron Scanning Primers
cM	centi Morgan
cm	centi meter
COV (XY)	covariance between character X and Y
CV	Coefficient of variation
DArT	Diversity Arrays Technology
DNA	Deoxyribo Nucleic Acid
DTF	Days to 50 per cent Flowering
EDTA	Ethylene Diamine Tetra Acetic Acid
ESTs	Expressed Sequence Tags
EST-SSR	Expressed Sequence Tagged-Simple Sequence Repeats
<i>Et al.</i>	and coworkers
EtBr	Ethidium Bromide
F ₂	Second filial generation
Fig	Figure(s)
g	Gram
GAM	Genetic advance as per cent of mean
Gbp	Giga base pairs
GBS	Genotyping by Sequencing
GCV	Genotypic coefficient of Variation
GFY	Green Fodder Yield
h	hour
h ² _(b)	Heritability in the broad sense
H ₂ SO ₄	Sulphuric acid

Abbreviation	Meaning
HCl	Hydrochloric acid
<i>i.e.</i>	That is
ICAR	Indian Council of Agriculture Research
IGFRI	Indian Grassland and Fodder Research Institute
ISSR	Inter-Simple Sequence Repeats
K	Selection differential at 5% selection intensity
kb	kilobase
Kg	Kilo gram
KMnO ₄	Pottasium permanganate
LG	Linkage Group
LOD	Logarithm of Odds
m	meters
M	Molar
m ²	Square meter
MAB	Marker-assisted breeding
MABC	Marker-assisted backcrossing
MARS	Marker-assisted recurrent selection
MAS	Marker Assisted Selection
mg	Milligram
MgCl ₂	Magnesium Chloride
min	Minute(s)
ml	Millilitre
mm	Millimeter
mM	Millimolar
MSE	Error mean square
ng	Nanogram
NGS	Next Generation Sequencing
NILs	Near Isogenic lines
nm	Nanometer
ns	Non-significant
°C	Degree Celsius
OD	Optical Density
PC	Principal Component
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PCV	Phenotypic Coefficient of variation
pH	Puissance de hydrogen (ion conc.)
PMN	Pearl Millet Napier
ppm	Parts per million
PVE	Phenotypic Variance Explained
QTL	Quantitative Trait Loci

Abbreviation	Meaning
r	Correlation coefficient
RAPD	Random Amplified Polymorphic DNA
RCBD	Randomized Complete Block Design
RFLP	Restriction Fragment Length Polymorphism
RILs	Recombinant inbred lines
RNA	Ribo Nucleic acid
rpm	Rotations per minute
RT	Room Temperature
sec	Second(s)
SNPs	Single Nucleotide Polymorphic
SSR	Simple Sequence Repeat(s)
STMS	Sequence Tagged Micro Satellite(s)
STS	Sequence Tagged Site
TAE	Tris Acetic Acid
Taq	<i>Thermus aquaticus</i>
Taq polymerase	<i>Thermus aquaticus</i> DNA polymerase
TBE	Tris Borate EDTA
TE	Tris EDTA buffer
T _m	Melting Temperature
Tris	Tris (hydroxy methyl) amino methane
UV	Ultraviolet
V	Volts
Var (X)	variance of the characters of X
Var (Y)	variance of the characters of Y
viz.	Namely
σ_p	phenotypic standard deviation

Bajra-Napier (BN) hybrid also known as king's grass is an interspecific manmade perennial grass. It is very popular among dairy farmers and is also a potential bioenergy crop due to its high biomass. It combines the unique features of both Bajra (*Cenchrus americanus*) and Napier (*Cenchrus purpureus*) species, which makes it more resilient to harsh environments with superior fodder quality (Ekemini *et al.*, 2012).

According to Harlan and deWet (1971), the sole species in the secondary gene pool is napier grass. This species exhibits considerable morphological variety in terms of characteristics including plant height, leaf size, hairiness, inflorescence size, colour, and stem thickness (Mannetje *et al.*, 1992). The prevalence of protogyny, which promotes interfecon, vegetative reproduction, and the perpetual growth trait, tends to keep the population heterozygous. It is a significant fodder and bioenergy crop since it is one of the most productive biomass plants in tropical and subtropical climates (Martha *et al.*, 2004; Strezov *et al.*, 2008). The most extensively grown species in the genus, however, is pearl millet (Bajra), which is related to Napier grass.

Napier is originated from the South Africa and was introduced to India and USA in 1912 and 1913, respectively. It is valued for its high biomass, perenniality and pest resistance. However, due to its undesirable quality attributes such as hard and fibrous stem, coarse leaf sheath and leaf blade along with serrated leaf margins, it is less preferred by livestock's. On the contrary, pearl millet is originated from West Africa (Gadaga *et al.*, 1999), it has high palatability with desirable traits like high biomass quality, non-shattering seed nature, drought tolerance and it grows well on low fertility soils (Serraj *et al.*, 2005). The development of interspecific triploid hybrids (commonly known as Bajra Napier Hybrids) and the introgression of beneficial alleles by traditional plant breeding are made possible by the sexual compatibility between Napier grass ($2n = 4x = 28$) and pearl millet ($2n = 2x = 14$) (Burton, 1944).

Bajra Napier Hybrids, also known as BN Hybrids, PMN Hybrids, King's grass, Elephant millet, and Cumbu-Napier Hybrids, are perennial hybrid grasses that grow tall (200–300 cm), upright, robust, and have deep roots. It is a Bajra and Napier grass F1 hybrid. One such tropical fodder crop that efficiently transforms solar energy into plant biomass and animal nutrition is BN Hybrid. BN hybrid grasses provide the most green

fodder per unit of time and space among all grasses. It reacts to strong nitrogenous fertilisation and is robust, nourishing, succulent, and tasty (Mukherjee *et al.*, 2020).

BN Hybrid contains about 10.2 per cent crude protein, 30.5 per cent crude fibre and is juicer and succulent at all stages of growth and hence the herbage is palatable. BN Hybrid is having more advantages over the parents like its perennial nature, quick growth, very productive and convenient for hay and silage making, edible young leaves and shoot which can be cooked to make soups and stews, more palatable leaves which are easily digestible, can be intercropped with various fodder legumes, used as mulch to prevent soil erosion, suitable as trap crop to fight against stem borer, used as wind breaks and production of biofuel, simple in cutting because of soft stem, soil restoring crop and improves fertility of the soil, resistant to drought due to deep root system, protects the other field crops from heat stress if planted in bund areas etc., (Kumari *et al.*, 2017; Mukherjee *et al.*, 2020).

BN hybrid is mostly grown in the regions of Asia, Africa, Southern Europe and America (Boonman, 1993; Gate *et al.*, 2018). It has the characteristics inherited from both Bajra and Napier parents viz., high number of tillers, soft leaves, vegetative propagation, resistance to several diseases, high nutritional quality and provide forage throughout the year (Jauhar, 1981; Tiwana, 2010). These hybrids proved to be superior to both the parents in terms of yield and quality. These interspecific hybrids are generally more acceptable as forage plants than elephant grass (Jauhar, 1981). The interspecific triploid hybrid between Bajra and Napier grass can have both the high dry matter production of Napier grass and the pearl millet-like feed quality. Even though the pearl millet parent is an inbred, the triploid hybrids produced by the interspecific cross are typically extremely diverse due to the heterozygosity of Napier grass (Hanna *et al.*, 2016).

Since Napier ($2n=4x=28$) is an allotetraploid with A'A'BB genomes and Bajra is a diploid ($2n=2x=14$) species with AA genomes, the obtained interspecific BN hybrids is a triploid (AA'B genomes) with $2n = 3x = 21$ chromosomes (seven chromosomes from Bajra and 14 chromosomes from Napier grass). A genome of Bajra is homologous with A' genome of Napier grass (Barbosa *et al.*, 2003). In general, chromosomes pair as seven bivalents (AA' genome) and seven univalents (B genome). Due to irregular chromosomal segregation during meiosis, the resultant hybrid is sterile and no fertile seed set which causes major bottleneck for its widespread distribution. (Hanna *et al.*, 1984; Techio *et al.*,

2006). Therefore, the only way of propagation of these interspecific F₁ hybrids is through vegetative means *i.e.*, through rooted slips/stem cuttings which is a cumbersome, laborious and costly affair for farmers as well as for planting material providers. The bulky material incurs huge transportation cost, which is a major bottleneck and limitation in dissemination and widespread use of this highly potential fodder cum bioenergy crop (Rana *et al.*, 2023).

Looking at its potential and popularity among farmers, several researchers accomplished *in vitro* polyploidization for restoring its fertility (Gildenhuis and Brix, 1964; Hanna, 1981; Hanna *et al.*, 1984; Gonzalez and Hanna, 1984; Rajasekaran *et al.*, 1986; Diz and Schank, 1993; Abreu *et al.*, 2006; Faleiro *et al.*, 2016). However, till date no such seed producing hybrids were available. One of the major reasons for this may be the use of chromosomal doubling agents *in vitro*, which leads to occurrence of somaclonal variations, mixoploidy, irregularities, genetic and epigenetic alterations (Rauf *et al.*, 2006; Liu *et al.*, 2009; Temel and Gözükmizi, 2015; Sanglard *et al.*, 2017). Thus, some alternative should be accessed and, in this direction, the successful development of fertile/seed producing BN hybrids will have great implications in wide spread and distribution of BN hybrid in the farmer's field, could be helpful in meeting the green fodder deficit of the country and ultimately improves the nutritional security of livestock, humans and socio-economic status of the farmers. In this direction, ICAR-IGFRI, Jhansi has recently developed world's first fertile novel tetraploid seed producing Bajra-Napier hybrid (TBN-20-15) (Rana *et al.*, 2023).

However, since this fertile BN hybrid is first of its kind, so no such genetic and genomic resource are available in this crop. For initiation of any crop improvement programme, genetic and genomic resources are highly required. Hence, first and foremost requirement of any crop improvement programme is development of linkage map. In this direction, the present work is designed in such a way that these genetic and genomic resources will be made available for further improvement in this crop. In molecular breeding, molecular marker techniques are often used to create linkage maps, tag genes for Marker Assisted Selection (MAS), and analyse the intricate links between phenotypic characteristics and the genomic areas that govern them (Quantitative Trait Locus [QTL] study). It is only possible to identify QTLs for significant agronomic features in any crop if a well saturated linkage map is provided. The whole genome is thought to include Simple Sequence Repeats (SSRs), which might serve as markers for linkage and QTL mapping

investigations (Chahota et al., 2020; Gupta & Varshney, 2000). They are highly variable, co-dominant and are distributed ubiquitously throughout the genome (Hamm *et al.*, 2023; Gupta & Varshney, 2000). They are the most promising class of markers for the creation of saturation maps because to their high polymorphism rate, which results from high mutation rate along with random occurrence in the genome (Verma *et al.*, 2015). In a variety of crop species, notably Bajra (Supriya *et al.*, 2011) and Napier grass (Paudel *et al.*, 2018), they have been extensively employed to produce linkage maps. As a result, SSR markers were employed in this study to create the first-ever BN hybrid framework linkage map. Using an F₂ mapping population, the first-ever framework linkage map was utilised to map biomass-related characteristics in BN hybrids.

Keeping above considerations in view, the present investigation entitled as, **“Construction of framework linkage map and genetic dissection of biomass related traits in Bajra-Napier Hybrid”** was focused on the following objectives:

- I. Development of framework linkage map in BN hybrid
- II. Phenotyping of F₂ population for Biomass related traits
- III. Genetic analysis of biomass related traits in BN hybrid

CHAPTER – 2

REVIEW OF LITERATURE

The following headings have been used to review the literature in relation to various areas of the current investigation:

2.1 Genomic resources in Bajra and Napier grass

2.1.1 Molecular markers

2.1.2 Construction of Bajra and Napier linkage maps

2.2 Phenotyping of F₂ population for Biomass related traits

2.3 Genetics analysis of biomass related traits in BN hybrid

2.3.1 QTL mapping studies

The most widely studied interspecific hybrids in the genus *Pennisetum* (now as *Cenchrus*) are those between pearl millet and elephant grass (Muldoon and Pearson, 1979; Jauhar, 1981). The first documented man-made hybrid was performed by Burton in 1941 (Burton, 1944). They were later produced in India by Krishnaswamy and Raman in 1949 followed by other countries. The initial purpose of obtaining this hybrid was to combine the high forage quality, non-shattering seed nature, vigor, seed size, drought tolerance and disease resistance in Bajra, with the rust resistance, aggressiveness and perenniality of Napier grass. These interspecific hybrids are generally more acceptable as forage plants than elephant grass (Jauhar, 1981). Besides their use in livestock production (Schank and Diz, 1991), they possess great potential as a biomass energy crop due to their high dry matter production. However, due to their triploid genome, these interspecific hybrids are sterile in nature, which causes major bottleneck for its improvement via breeding programs.

To overcome this, Scientists at ICAR-Indian Grassland and Fodder Research Institute, Jhansi has developed World's first fertile tetraploid seed producing BN hybrid (Rana *et al.*, 2023). This hybrid is able to produce more than 15,000-20,000 seeds throughout the year with 80-90% seed germination ability. This fertile tetraploid BxN hybrid reported for the first time globally will be very helpful in easy and cost-effective dissemination of this highly potential forage crop to the farmer's field. It has the potential

to be the game changer in biofuel production, grassland rejuvenation programs besides bridging the fodder demand supply deficit.

2.1 Genomic resources in Bajra and Napier grass

The availability and ease of access to genomic materials is a prerequisite for using molecular breeding efforts in a particular crop species. Since, fertile tetraploid Bajra Napier Hybrid is recently developed by ICAR-IGFRI. Therefore, there are no well-established genomic resources in this crop. Hence, the parental genomic markers, genetic linkage maps, whole genomes or transcriptomes, EST resources, etc. were taken as reference. Some of them reported earlier have been discussed below:

2.1.1 Molecular markers

The earlier reports of molecular markers in Napier grass was found to be isozyme markers used for screening (Shenoy & Vasil, 1992). Afterwards DNA-based molecular markers were created in Napier using data from several bases in the DNA molecule, such as point mutations, indels (insertion, deletion), and mutation in the repetitive sequences, and they were used for a variety of reasons (Gupta & Varshney, 2000; Supriya *et al.*, 2011; Paudel *et al.*, 2018). An advantage of DNA markers over morphological and isozyme markers was the higher amount of polymorphism (Paterson *et al.*, 1991), which can be used to generate a saturated linkage map. Following that, PCR-based DNA markers like RAPD had been developed and employed for phylogenetic analysis, diversity analysis, and the determination of taxonomic connection among the Bajra and Napier grass species. (Babu *et al.*, 2009; Pedraza-Garcia *et al.*, 2010). A linkage map using RFLP markers was constructed in pearl millet (Liu *et al.*, 1994). Subsequently, both homologous and heterologous restriction fragment length polymorphism (RFLP) markers, and simple sequence repeats (SSRs) were used for the construction of linkage map of pearl millet (Qi *et al.*, 2004). Amplified fragment length polymorphism (AFLP) markers were used to screen the Napier grass accessions and provided potential heterotic groups for Bajra and Napier grass (Harris *et al.*, 2010). PCR-based molecular marker genetic map for pearl millet using a recombinant inbred line (RIL) population was developed by Pedraza-Garcia *et al.*, 2010. With the advantage of SSRs over the others, SSRs were developed in both Napier and Bajra also (Wang *et al.*, 2010; Dowling *et al.*, 2014; Yashveer *et al.*, 2018). Kandel *et al.* (2016) investigated to discover whether microsatellite markers between

Napier grass (*Cenchrus purpureus*) and pearl millet (*Cenchrus americanus*) could be transferred between the two species. Expressed Sequence Tag-Simple Sequence Repeat (EST-SSR) markers were used to screen the PMN hybrids and conserved Napier grass specific markers were identified (Dowling *et al.*, 2013). Besides above reported markers, other class of markers has also been developed in both Bajra and Napier such as Inter-Simple Sequence Repeat (ISSR) markers, which are amplified with SSR-anchored primers, were also used for the characterization of Bajra and Napier grass (Pedraza-Garcia *et al.*, 2010; Jade *et al.*, 2021). RAPD and ISSR markers were used for the diversity study of Napier grass collections (Babu *et al.*, 2009).

In Bajra and Napier grass, a more sophisticated marker technique has recently been used. The Napier grass genome was initially surveyed using next-generation sequencing by Paudel *et al.* in 2018. The genomes of the pearl millet and napier grass demonstrated significant levels of synteny in the sequences, as well as expansions and genomic reorganisation between the two genomes. Genotyping by sequencing (GBS) was done to generate high density SNP markers and also to select the candidate genes for forage traits in Bajra (Mukhtar *et al.*, 2019). For understanding genomic data and for development of SSR markers in Napier grass species, next generation sequencing technologies were used on the transcriptome of Napier grass (Wang *et al.*, 2018). A total of 12,744 EST-SSRs have been developed, of which 200 were validated among 20 Napier accessions for diversity studies.

Likewise Single Nucleotide Polymorphic (SNPs) markers were utilised for the construction of linkage map in Bajra (Palumbo *et al.*, 2019). High-throughput and low-cost Diversity Arrays Technology (DArT) markers were also used for the enhancement of pearl millet molecular linkage map density (Supriya *et al.*, 2011). This array can be advantageous for mapping, association studies and analysis of variation in a large collection of lines.

2.1.2 Construction of Bajra and Napier linkage maps

Recently, genetic linkage maps have established themselves as essential tools for both applied plant breeding and fundamental genetic study. Linkage maps have made it possible to identify tightly connected DNA tags for use as diagnostic tools in plant breeding and aided in the identification of DNA markers associated to specific genes of

significant agronomic value. Quantitatively inherited traits has been characterized by the use of linkage maps, including identifying the genomic regions containing contributing loci, postulating the potential types of gene action, and figuring out the function of epistatic effects in phenotype specification (Tanksley, 1993). Linkage maps based on molecular markers also have the ability to close the knowledge gap between organismal biochemistry and physiology and understanding of phenotype based on genetics (Gilpin *et al.*, 1997). To identify and differentiate QTL areas in the genome, a comprehensive linkage map is necessary. Tightly connected markers may be verified for use in marker-assisted selection (MAS) when key QTLs have been deciphered, and they may even serve as a starting point for the positional cloning of the underlying functional resistance gene(s) (Haley and Andersson, 1997).

Various linkage maps has been developed in Bajra using molecular markers systems ranging from RAPDs, ISSRs, SSRs to DArT, SNPs, STs and CISPs (Liu *et al.*, 1994; Qi *et al.*, 2004; Pedraza-Garcia *et al.*, 2010; Supriya *et al.*, 2011; Sehgal *et al.*, 2012; Rajaram *et al.*, 2013; Vengadessan *et al.*, 2013; Moumouni *et al.*, 2015; Punhuri *et al.*, 2016; Durgaraju *et al.*, 2017; Saritha *et al.*, 2017; Maganlal *et al.*, 2018). However, only one linkage map has been developed in Napier using SNPs technology (Paudel *et al.*, 2018). The list of previously published maps including the most advanced map of Bajra and Napier has been depicted in Table 2.1 mentioning type of marker system along with mapping population they have used.

Table 2.1: List of linkage map constructed in Bajra and Napier using DNA based molecular markers

Cross type	Mapping Population	Markers mapped	No of linkage groups	Distance (cM)	Average marker density (cM)	Reference
Bajra						
Intraspecific	F ₂	181 RFLPs	7	303	2	(Liu <i>et al.</i> , 1994)
Intraspecific	F ₂	353 RFLPs and 65 SSRs	7	472.6	1.9	(Qi <i>et al.</i> , 2004)
Intraspecific	RILs	196 (66 SRAPs, 63 RAPDs, 27 ISSRs, 31 bajra, 6	7	1,796	9.2	(Pedraza-Garcia <i>et al.</i> , 2010)

		sorghum & 3 maize SSRs				
Intraspecific	RILs	574 DArTs	7	1,148	0.28	(Supriya <i>et al.</i> , 2011)
Intraspecific	F ₂	133 SSRs, SNPs & CIPs	7	815.3	6.13	(Sehgal <i>et al.</i> , 2012)
Intraspecific	F ₂	99 new EST-SSRs, 17 previously mapped EST-SSR, 53 genomic SSRs & 2 STS	7	615	5.9	(Rajaram <i>et al.</i> , 2013)
Intraspecific	F _{2:3}	40 SNPs, 36 SSRs, 28 EST-SSRs & 5 STS	7	1,019	23	(Vengadessan <i>et al.</i> , 2013)
Intraspecific	F ₂	3321 SNPs & 19 SSRs	7	640	0.51	(Moumouni <i>et al.</i> , 2015)
Intraspecific	RILs	16,650 SNPs	7	716.7	23.23	(Punnuri <i>et al.</i> , 2016)
Intraspecific	RILs	39 Genomic SSRs and 49 EST-SSRs	7	725.5 (Haldane units)	9.8	(Durgaraju <i>et al.</i> , 2017)
Intraspecific	RILs	39 SSRs	7	536.8	13.7	(Saritha <i>et al.</i> , 2017)
Intraspecific	F ₂	27 SSRs	7	248.37	9.19	(Maganlal <i>et al.</i> , 2018)
Napier						
Intraspecific	F ₁	1,913 SNPs	14	1,410	0.73	(Paudel <i>et al.</i> , 2018)

2.2 Phenotyping of F₂ population for Biomass related traits

Development of lines having high green fodder yield (GFY) along with best qualitative traits are the main manifest of the forage breeder. Identification and development of best BN hybrid must have promising traits viz., high GFY and good quality of palatability. Keeping this in view, the BN hybrids are evaluated on the basis of morphological and quality traits, however these traits are influenced by the environmental factors such as climatic condition, soil and different agronomic practices. Therefore,

suitability of the specific hybrid for particular traits should be known. Several study of Pearl millet, Napier and BN hybrids for morphological traits were studied by the researchers and are as follows:

Based on GFY, DMY, CP per cent and *in vitro* DM digestibility (IVDMD), two pearl millet by Napier hybrids hybrids were selected by Gupta & Mhere, 1997. To study variation among the different morphological traits viz., number of tillers per plant, number of leaves per tiller, plant height, leaf length and green forage yield Turano *et al.* (2016) analyzed six different Bajra Napier hybrids. There was positive correlation of GFY with plant height and leaf breadth. Hence, direct selection of these traits increases the green fodder yield.

The genetic advance (GA) and broad sense heritability (h^2_{bs}) for the traits viz., plant height, leaves per plant, tillers per plant, leaf length, leaf to stem ratio, leaf width, green forage yield, dry matter yield, acid detergent fiber, crude protein yield and oxalate content showed that the selection of these traits for developing frost tolerant BN hybrid was effective (Kumari *et al.*, 2017). Further, correlation and path coefficient study among 18 BN hybrid genotypes revealed that traits viz., leaf length, leaves per plant, dry matter yield (DMY), plant height and crude protein (CP) yield per plant are important for effective selection for the development of high forage yield BN hybrids (Kumari *et al.*, 2017).

Further, Singh *et al.*, (2020) studied the morpho-physiological and GFY under different salt stress condition in pot conditions. The GFY was positive correlated with leaf area, number of leaves and plant height, whereas negative correlated with number of tillers per plant.

Similarly, Ahamed *et al.*, 2021 compared two Napier cultivars, in terms of forage growth, biomass yield, and nutritional quality. The effects of cultivar and cutting period were significant on plant height, leaves per tiller, leaf DM production and leaf stem ratio (LSR). Likewise, Ekemini *et al.*, 2012 evaluated forage potentials of interspecific hybrids between elephant grass selections and cultivated pearl millet genotypes of Nigerian origin and found that significant differences was observed between harvest intervals, plant height and dry matter content except dry matter yields. Dry matter content showed negative

correlation with plant height while dry matter yields showed positive correlation with plant height.

Recently, Sinche *et al.*, 2021 has developed a Napier grass F₁ mapping population (also known as segregating pseudo F₂ population) by hybridizing genetically distant parents with contrasting flowering-time- and biomass-related traits for characterization of flowering time and biomass related traits. They have observed near-normal distributions for flowering date, plant height, number of tillers, stem diameter and leaf width confirming the quantitative nature of these traits and suggested that these pseudo F₂ population can be used as excellent source for identifying QTLs and candidate genes for flowering time and biomass related traits. Meanwhile, Sarker *et al.*, 2021 evaluated the performance of Napier cultivars in terms of forage yield, plant morphology and nutrient contents under two different agro-ecology and geo-topographic conditions.

In addition Bajra and Bajra-Napier hybrid contain oxalate content within safe limits, however, they may prove toxic if fed over for extended period of time. Oxalates react with calcium to produce insoluble calcium oxalate, reducing calcium absorption. This leads to a disturbance in the absorbed calcium: phosphorus ratio, resulting in mobilization of bone mineral to alleviate the hypocalcemia. Prolonged mobilization of bone mineral results in nutritional secondary hyperparathyroidism or osteodystrophy fibrosa. Here for genotypic selection, oxalate content should be considered and it should be within safe limit *i.e.*, <20% (Rahman and Kawamura, 2011).

2.3 Genetics analysis of biomass related traits in BN hybrid

2.3.1 QTL mapping studies

The identification and localization of genes in the genome which control variation for quantitative traits can greatly facilitate their selection in breeding programs. Thoday (1961) demonstrated that simply inherited gene markers can be used as tags to locate quantitative trait loci (QTLs). The technique for identification of QTL by gene markers became more efficient with the availability of molecular markers.

Molecular markers have been used to identify QTLs in several crops such as maize (Almeida *et al.*, 2012; Liu *et al.*, 2012), wheat (Cuthbert *et al.*, 2008; Rebetzke *et al.*, 2008; Bennett *et al.*, 2012), rice (Steele *et al.*, 2012; Wei *et al.*, 2012), tomato (Tanksley *et al.*,

1982; Paterson *et al.*, 1988), common bean (Kwak *et al.*, 2008; Pérez-Vega *et al.*, 2010), soybean (Abdel-Haleem *et al.*, 2011; Zhang *et al.*, 2012) and chickpea (Anbessa *et al.*, 2009; Gowda *et al.*, 2011).

Since fertile tetraploid Bajra Napier Hybrid is first of its kind, therefore no QTL is reported so far for any trait. Hence, QTLs reported for Bajra and Napier were taken as reference. In bajra, QTLs were reported for 50% Flowering Time, Plant Height, Panicle Length and Thousand Grain Weight by Kumar *et al.*, 2021 (Table 2.2) while, for Napier QTLs were reported for Acid Detergent Fiber, Acid Detergent Lignin, ash, Dry Matter, *In Vitro* Organic Matter Digestibility, Neutral detergent Fibre and Organic Matter content by Muktar *et al.*, 2022 (Table 2.3).

Table 2.2: Molecular markers closely associated with desirable traits in Bajra for use in marker-assisted selection

Trait mapped	QTL/gene	Associated markers	Reference
50% Flowering Time (FT)	1/122	pgpb10307-pgpb8836	Kumar <i>et al.</i> , 2021
Plant Height (PH)	1/120	Xipes146-pgpb7504	
	2/24	Xipes060-pgpb8259	
	2/48	pgpb6832pgpb11452	
	3/30	pgpb12310-pgpb7799	
	3/80	pgpb12310-pgpb7799	
	4A/42	pgpb12326-pgpb11246	
	4A/124	pgpb6062-pgpb7101	
	4A/142	pgpb13161-pgpb11560	
Panicle Length (PL)	1/60	pgpb9130-pgpb6955	
	5/24	pgpb10025-Xipes094	
	E/0	Xipes024-pgpb10727	
Thousand Grain Weight (TGW)	3/32	pgpb7799-pgpb7379	
	7/60	pgpb10822-Xipes198	

Table 2.3: Molecular markers closely associated with desirable traits in Napier for use in marker-assisted selection

Trait mapped	Associated chromosome	Associated markers	Reference
ADF	A02 A07	IG100036297 IG100007552	Muktar <i>et al.</i> , 2022
ADL	B02 A04	IG100310013 M48572_D23546094	
ash	A01 B02	D23592459 IG100039072	
DM	A01 B05	IG100294731 IG100107307	
IVOMD	A02 A04	IG100036297 D30280676	
NDF	B03 B04	D23601192 IG100092231	
OM	B01 B02	D23572727 D23634207	

ADF = Acid Detergent Fiber, **ADL** = Acid Detergent Lignin, **DM** = Dry Matter, **IVOMD** = *In Vitro* Organic Matter Digestibility, **NDF** = Neutral detergent Fibre, **OM** = Organic Matter

CHAPTER – 3

MATERIALS AND METHODS

The present investigation entitled “**Construction of framework linkage map and genetic dissection of biomass related traits in Bajra-Napier Hybrid**” was carried out using immortalized F₂ population at Central Research Farm, Crop Improvement Division, ICAR-IGFRI, Jhansi, Uttar Pradesh. The genotypic work including DNA isolation, purification, amplification, polymorphism survey and genotyping were carried out at Central Biotechnology lab, Crop Improvement Division, ICAR-IGFRI, Jhansi, Uttar Pradesh. The material used and the methodology adopted to achieve the objectives of the investigation are given here under:

3.1 Climate

3.2 Plant Material

3.3 Experimental details

3.4 Phenotypic characterization

3.5 Statistical methodology

3.5.1 Analysis of Variance

3.5.2 Estimation of mean performance

3.5.3 Estimation of variability parameters

3.5.4 Correlation coefficient analysis

3.5.5 Cluster and Principal component analysis

3.6 Genotypic characterization

3.7 Genotypic analysis

3.1 Climate:

Climate of the Bundelkhand region is sub-tropical with cool winter, sweltering summer and semi-humid weather. The average annual rainfall of this region varies from 850-1000 mm. Jhansi had an average temperature of 25.8⁰C and annual average precipitation of about 870 mm. Jhansi experiences extreme temperature due to its location on a rocky and plateau region. The geographic coordinates for Jhansi were 33°59'12"N longitude and 74°47'52"E latitude at an altitude of 1640m above mean sea level. The research unit falls under the sub-tropical climatic zone.

3.2 Plant material

An immortalized F₂ population of 250 individuals derived from an intraspecific cross of *Cenchrus americanus* and *Cenchrus purpureus* was used for the construction of genetic linkage map. For immortalized F₂ development, rooted slips from a single F₂ plant were harvested and transplanted through vegetative means. The parents were different from one another in terms of a variety of agro-morphological characteristics. The immortalized F₂ were grown in Central Research Farm, ICAR-IGFRI for collecting leaf samples for DNA extraction and also for phenotyping work.

3.3 Experimental details:

Location	: Central Research Farm, Division of Crop Improvement, ICAR-IGFRI, Jhansi
Cropping season	: <i>Kharif, Rabi and Summer</i> (2022-23)
Experimental design	: Augmented RCBD
Date of sowing	: 22 nd July, 2022
Spacing (Row to row & plant to plant)	: 1.5 meter x 1.0 meter
Inter block distance	: 2 meter
Total number of blocks	: 08
No. of rows per plot	: 01
Row length	: 5.0 meter
Total number of lines	: 252
No. of checks (parents)	: 02 (Tetra Bajra, FD-475)
No. of F₂ individuals	: 250

3.4 Phenotypic Characterization

3.4.1 Phenotyping of immortalized F₂ lines

A population of 250 immortalized F₂ lines were phenotyped for 22 biomass related traits. The immortalized F₂ lines were evaluated at Central Research Farm, ICAR-IGFRI, Jhansi. The plants were grown in a single row having row to row distance of 1.5 meter and

plant to plant distance of 1.0 meter in an augmented randomized complete block design (RCBD) in 8 blocks. The block to block distance is 2.0 meter. The recommended agronomic practices were followed during the crop growth period.

3.4.1.1 Measurement of morphological traits

Plant height (PH), Leaf length (LL), Leaf Width (LW), No. of tillers per plant (NTPP), Stem Thickness (ST), Inter Nodal Length (INL), No. of Leaves per Plant (NLPP) and Spike Length (SPL) were important morphological traits to characterize genotypes for biomass related traits. All these parameters were recorded at 50% flowering stage of the crop. The main tillers of the each plant were taken for readings. The detailed description of other traits has been provided in Table 3.3.

3.4.1.2 Measurement of Phenological traits

Days to 50% Flowering (DTF) is one of the important phenological trait to be measured for biomass quantification. Data were collected on days from transplanting to 50% flowering by calculating the difference of days from date of transplanting to the date when 50% of the plants in a line showed the first fully open flower.

3.4.1.3 Measurement of physiological traits

Chlorophyll content

The total chlorophyll concentration was measured by non-destructive method using chlorophyll concentration meter (Model- MC-100 make Apogee Instruments) and it was calibrated to measure chlorophyll concentration in leaves with units of μmol of chlorophyll per m^2 area. Observations were recorded by selecting five healthy leaves from the row during a sunny day.

3.4.1.4 Measurement of anti-nutritional factor(s)

Determination of oxalate content

a. Materials Required:

Oxalic acid, Potassium permanganate solution, 1.0 M sulfuric acid, Chemical balance, Burette, Burette stand, Pipette, Conical flask, Funnel, Measuring flask, Weighing bottle, White tile, Burnet, Wire gauze

b. Apparatus Setup:

1. In burette – KMnO_4 solution
2. In Conical flask – 10ml of oxalic acid + Sulfuric acid
3. Indicator – Self indicator (KMnO_4)
4. End Point – Appearance of permanent pale pink colour

c. Procedure

The oxalate content was determined from the modified titration procedure described by Unuofin *et al.*, 2017. About 1 g each of the pulverized plant samples was weighed in a conical flask and 75 ml of 3M H_2SO_4 was added and agitated on a magnetic stirrer for one hour. The mixture was filtered and 25 ml of filtrate collected was heated to about 90°C . The hot aliquot was titrated uninterruptedly against 0.05 M KMnO_4 till a light pink colour change which lasted for 15 s was observed. This marked the endpoint of titration. The titre value of each of the plant sample extracts was multiplied by 2.2 mg of oxalate taken as equivalence of 0.05M of KMnO_4 used for titration.

d. Calculations

$$\text{Oxalate content (\%)} = 2.2\text{mg} \times \text{titre value} \dots\dots\dots$$

3.5 Statistical methodology:

The experimental data collected in respect of various characters on 250 Bajra Napier Hybrid immortalized F_2 lines along with two parental checks during 2022-23 and for 22 traits were compiled by taking the mean values of selected plants in each plot and subjected for following statistical analysis. The data was statistically analyzed through R package and statistical software StatistiXL version 2.0. For analysis of data, details of statistical methods adopted are given below:

3.5.1 Analysis of variance

The evaluation of the experiment during 2022-2023 was conducted in an augmented block design (Federer and Raghavarao, 1975; Peterson, 1985). For the examination of a high number of breeding lines, this design is critical. The test entries are planted only once in this design, with no replication *i.e.*, each block has a unique set of

unreplicated entries, whereas a set of checks (c) is repeated in each block to estimate the error mean square and block impact. Randomization is done in such a way that all the checks and a part of test genotypes fall only once in each block. An equal number of test genotypes was planted in each block to facilitated statistical analysis. In this design, the values of genotypic means are adjusted for the block effect, which are measured by the check entries which present in each block. The block size is determined by the number of blocks (b), the number of check entries (c) and the number of test genotypes/entries (v).

When the block size is constant the following relationship hold true:

$$N = bc + v = b(c + n) = \text{total number of plots}$$

Where,

b = number of blocks,

v = number of genotypes

P = c+n = number of plots per block

c = number of check varieties

n = v/b number of genotypes per block

The total number of blocks is determined by the need to have at least 10 degree of freedom for error in the analysis of yield data which is given by the following relationship:

$$b > [(10/c-1) + 1]$$

In present investigation, 8 blocks were used with two checks and 250 test entries. The analysis of variance for augmented design was done using the method given by Federer (1956, 1961). The details of the statistical methods adopted for data analysis are presented below:

Checks	Blocks			Total	Mean
	1	2.....	4		
1.	$X_{1,1}$	$X_{1,2} \dots$	$X_{1,1}$	C_1	X_1
2.	$X_{2,1}$	$X_{2,2} \dots$	$X_{1,1}$	C_2	X_2
3.	$X_{3,1}$	$X_{3,2} \dots$	$X_{3,4}$	C_3	X_3
c.	B_1	B_2	B_4	G	M

Where,

X_{ij} = any observation of i^{th} check in j^{th} block

$B_j = \sum_i^c X_{ij}$ = Sum of all checks in j^{th} block

$C_i = \sum_j^B X_{ij}$ Sum of any observation of i^{th} check in overall blocks

$G = \sum_j^B X_{ij}$ = Grand total of all checks

$X_i = C_i/b$ = Mean of the i^{th} check

$M = \sum X_i = G/b$ = Sum of all check means b = number of blocks

c = number of checks

Table 3.1: Analysis of variance (ANOVA) for Augmented Block Design

S. No.	Source of Variation	d.f.	SS	MSS	F Value
1	Blocks	b-1	SSb	MSSb	MSSb/MSSE
2	Entries	e-1	SSe	MSSe	MSSe/MSSE
A	Genotypes	g-1	SSg	MSSg	MSSg/MSSE
B	Check	v-1	SSv	MSSv	MSSv/MSSE
3	Genotypes/ Check	[g+(v-1)]	SSgv	MSSgv	MSSgv/MSSE
4	Error	(b-1)(v-1)	SSE	MSSE	
	Total	N-1	TSS		

Where,

$$SST = \sum \sum_j X_{ij}^2 - G^2/bc$$

$$SSB = \frac{1}{c} \sum_j B_j^2 - G^2/bc$$

$$SSC = \frac{1}{b} \sum_i C_i^2 - G^2/bc$$

$$MSS = SST - (SSB + SSC) \quad MSE = SSE / (b-1)(c-1)$$

Then compute block effect r_j for each block where $r_{ij} = \frac{1}{c} (B_j - M)$

After this, a table of adjusted yield for all the genotypes was constructed.

Where,

b = Number of blocks

e = Number of total entries

g = Number of germplasm accession tested

d.f. = Degree of freedom

SSb = Sum of Square due to blocks

SSv = Sum of Square due to checks

SSg = Sum of square due to genotypes/accessions

SSE = Sum of Square due to error

MSS = Mean sum of square

MSSE = Mean sum of square due to error the significance was tested by referring to the "F" table value given by Fisher and Yates (1963)

Y_{ij} = Yield of the i^{th} genotype in j^{th} block

$Y_i = Y_{ij} - r_j$ = yield of the i^{th} variety adjusted for block differences Variances for different pair wise comparisons:

1. Difference between two check means = $2\text{MSE}/b$
2. Difference between adjusted yield of two genotypes in the same block = 2MSE
3. Difference between adjusted yield of two genotypes in different blocks = $2\text{MSE} [1 + \frac{1}{c}]$
4. Difference between adjusted yield of genotypes and check means = $2\text{MSE} (b+1)(c+1)/bc$

Least significant difference values:

1. For two check means = $t \alpha \sqrt{2\text{MSE}/b}$
2. For two adjusted genotypes in same block = $t \alpha \sqrt{2\text{MSE}}$
3. For two adjusted genotypes in different blocks = $t \alpha \sqrt{2\text{MSE} (c + 1)/c}$
4. For an adjusted genotype against check means = $t \alpha \sqrt{2\text{MSE} (b + 1)(c + 1)/bc}$

For all L.S.D. the t values are two tail value at α % level of significance and $(b-1) (c-1)$ degrees of freedom.

3.5.2 Estimation of mean performance

3.5.2.1 Mean: The average values of each character was estimated by dividing sum of the observations by the number of characters.

$$\text{Mean (x)} = \frac{\sum x}{N}$$

Where,

$\sum x$ = sum of all observation for each character in each replication

N = number of observations

3.5.2.2 Range: It was taken as the difference between the highest and lowest mean value for each character.

$$\text{Range} = [X_n - X_1]$$

Where

X_n = highest mean value of the character

X_1 = lowest mean value of the character

3.5.2.3 Coefficient of variation (%)

$$CV = \frac{\sqrt{MSE}}{\bar{X}} \times 100$$

Where,

$\bar{X}$ = general mean of character and the Coefficient of variation (CV), being a unitless measurement is a good basis for comparing the extent of variation between different characters with different scales.

3.5.2.4 Standard error

The standard error of the difference between any two treatments is expressed by the following formula:

$$SEd = \sqrt{2MSE/r}$$

Where,

MSE = error mean square and

r = number of replications.

The significance of difference among the treatments means was tested by F test at 5% and 1% level of significance. Whenever, the F value was found to be significant; critical difference was calculated to test the significance of difference between any two treatment means as follows:

$$C.D. (at 5\%) = SEd \times t (5\%) \text{ at error degrees of freedom}$$

3.5.3 Estimation of variability parameters

Burton and Devane (1953) proposed a method for assessing genotypic and phenotypic coefficients of variability for all traits. The Genotypic and phenotypic variance were calculated following standard procedures given below:

3.5.3.1. Phenotypic variance

$$\sigma^2 (p) = \sigma^2 (g) + MSSE$$

3.5.3.2. Genotypic variance

$$\sigma^2 (g) = \frac{MSSV - MSSE}{r}$$

Where,

$\sigma^2(p)$ = Phenotypic variance

$\sigma^2(g)$ = Genotypic variance

MSSV = Mean sum of square of treatment/accessions (adjusted)

MSSE = Mean sum of the square due to error

The test of significance was carried out using the 'F' table value of Fisher and Yates (1963).

3.5.3.3 Coefficient of variation

The phenotypic (PCV) and genotypic (GCV) coefficients of variation were determined using Burton and Devane's standard approach (1953) which are given below:

a) Phenotypic coefficient of variation (PCV):

$$\text{PCV (\%)} = \frac{\sqrt{\text{phenotypic variance (Vp)}}}{\text{The general mean of a population (GM)}} \times 100$$

b) Genotypic coefficient of variation (GCV):

$$\text{GCV (\%)} = \frac{\sqrt{\text{Genotypic variance (Vg)}}}{\text{The general mean of a population (GM)}} \times 100$$

The categories for genotypic (GCV) and phenotypic coefficients of variation (PCV) given by Sivasubramanian and Menon, (1973) as low (0-10%), moderate (10-20%), and high (20% and above) were adopted in the present investigation also.

3.5.3.4 Heritability in the broad sense ($h^2_{(b)}$)

Heritability in a broad sense was calculated by using the formula suggested by Allard, (1960).

$$\text{Heritability (h}^2 \text{ \%)} = \frac{\text{Genotypic variance (Vg)}}{\text{Phenotypic variance (vp)}} \times 100$$

Heritability in the broad sense ($h^2_{(b)}$) was estimated as the ratio of genotypic variance to phenotypic variance (Allard 1960). Where, $p^2\sigma$ and $g^2\sigma$ are the phenotypic and genotypic variances of the character and $\bar{X}$ is the mean of the character.

The estimates of broad sense heritability ($h^2_{(b)}$) were also classified into three categories as suggested by Robinson *et al.*, (1949).

3.5.3.5 Genetic advance

Improvement in the mean genotypic value of selected plants over the parental population is known as genetic advance. It is the measure of genetic gain under selection.

$$\text{Genetic advance} = h^2_{(b)} \times \sigma_p \times K$$

Where,

$h^2_{(b)}$ = heritability in broad sense,

σ_p = phenotypic standard deviation, and

K = selection differential at 5% selection intensity

The value of K (at 5% selection intensity) = 2.06 was adopted following Allard (1960). Genetic advance expressed as percent of population mean was also calculated as per formula mentioned below:

$$\text{Genetic advance as \% of mean} = \frac{\text{Genetic advance}}{\text{General mean population (Gm)}} \times 100$$

Genetic advance as percent mean was also classified into 3 categories viz., low (0- 10%), moderate (10-20%), and high (20% and above) as suggested by Johnson *et al.*, (1955).

3.5.4 Correlation coefficient analysis:

Across the genotypes, the simple correlation coefficient was calculated to determine the direction and magnitude of association among different characters. Simple correlations were computed by using the formula given by Singh and Narayanan (1993):

The correlation coefficient (r) between x and y characters can be estimated as the Genotypic correlation coefficient between characters X and Y.

$$r_{xy} = \frac{COV(XY)}{\sqrt{Var(X) \times Var(Y)}}$$

Where,

r = correlation coefficient

COV (XY) = covariance between character X and Y

Var (X) and Var (Y) = variance of the characters of X and Y, respectively.

The significance of the correlation coefficient was tested at an appropriate level of significance. The significance values of correlation coefficient (r) were tested at (n-2) degree of freedom, where 'n' is the number of genotypes/accessions.

3.5.5 Cluster and principal component analysis

Cluster analysis was done to deduce the genetic similarity/dissimilarity between the lines under study and was performed using the R software. Pearson (1901) was the first to define the principle component analysis (PCA) approach, while Hotelling (1933) describe the computation method. PCA is a multivariate statistical approach that uses a linear combination of variables to reduce data with a large number of associated variables into a much smaller set of new variables, accounting for the majority of the variation in the original variables. The output for interpretation and grouping of various morphological parameters with respect to PCA and cluster analysis was performed using the statistical software StatistiXL version 2.0 and R software respectively.

3.6 Genotypic Characterization

3.6.1 Extraction of plant genomic DNA

Genomic DNA was isolated from young leaf tissues (100mg) of the parents and F₂ individuals using standard kits (Chromous biotech, India). The leaf tissues were rinsed in deionized water, dried on tissue paper discs and ground to fine powder in liquid nitrogen in autoclaved pre-cooled pestles and mortars. The ground tissue was added with 1.0 ml extraction buffer and mixed with pipette having cut tip. That extract was transferred to a separate 2.0 ml centrifuge tubes and spun at 10,000 rpm for 10 min. The clear lysate was loaded onto the spin column. A mini-spin column was placed into the 2.0 ml collection tube and 600 µl of lysate was loaded onto it each time. The column was spun at 10,000 rpm for 1.0 minute at room temperature. The contents of the collection tube were discarded and the spin column was placed back in the collection tube. Binding buffer of 500 µl was added to the column and spun at 7,000 rpm for 1.0 minute at room temperature. The contents were discarded from the collection tube and the spin column was placed back into the same collection tube. Wash buffer of 600 µl was added to the column and spun at 7,000 rpm for 1.0 minute at room temperature two times followed by regular removal of the contents from collection tube. The empty column was spun at 10,000 rpm for 3.0 minutes at room temperature. The spin column was placed in a fresh 1.5 ml vial. Elution buffer of 50 µl was added into the center of the spin column membrane. The vial along with the spin column was spun at room temperature for 1.0 minute at 7,000 rpm. Again 50 µl of elution

buffer was added followed by the spin at 7,000 rpm for 1.0 minute at room temperature. The spin column was discarded and the vial containing genomic DNA was stored at 0°C.

3.6.2 Quantification of genomic DNA

DNA concentration was checked by agarose gel electrophoresis. All the DNA samples were electrophoresed on 0.8 % agarose gels in 1X TBE buffer (pH 8.0) with known concentration of uncut λ DNA. The gel was stained in ethidium bromide solution in a final concentration of 10 $\mu\text{g/ml}$ and scanned in a gel documentation system (Syngene, UK). The concentrations of DNA samples were compared with the uncut λ DNA and diluted to make final working DNA solution having concentration of 13 ng/ μl .

3.6.3 Quality of DNA

Quality of the DNA (*i.e.*, approximate purity of DNA) was estimated by determining the ratio of absorbance at 260 and 280 nm using Nanodrop.

$$\text{Quality of DNA} = \text{OD at 260nm} / \text{OD at 280nm}$$

Good DNA preparation exhibited the following spectral properties: $A_{260}/A_{280} = 1.8$ to 2.0

The presence of RNA and protein is indicated by higher and lower ratios, respectively. The ratio of A_{260}/A_{280} absorbance comes 1.8 for pure double stranded DNA. If the ratio for any sample is more than 1.8, the sample has RNA contamination; if the ratio is less than 1.8, the sample has protein contamination. The samples that deviated from this ratio were purified further.

3.6.4 SSR marker studies

Standardization of SSR-PCR procedure

PCR protocol was standardized for carrying out amplification using SSR primers. Reaction conditions and optimum concentration of various components *viz.*, Taq buffer, template DNA, Taq DNA polymerase and primer were standardized. 10 μl of mixture for PCR analysis for the respective studies was prepared as follows:

To prepare the reaction volume, different amounts of each reaction component in ' μl ' were used. Using sterile distilled water, the final volume was make up to 10 μl completed. In a 1.5 ml centrifuge tube, the reagents were well combined and vortexed for a few seconds. This mixture was evenly placed into each 0.2 ml thin-walled PCR reaction tube (Axygen

Scientific Pvt. Ltd, New Delhi, India), and 2.0 µl of DNA was added to each tube individually. For cyclic amplification, the tubes were put in a thermal cycler (BIOER XP Cycler and Biometra Advanced).

Table 3.2: List of components used in PCR reaction

S. No.	Reagents	Quantity
1.	Template DNA (13 ng/µl)	2.0 µl
2.	Primer	1.0 µl (Forward and Reverse primer each)
3.	Master mixture (Hi media)	3.5 µl
4.	Sterile distilled water	3.5 µl (final volume 10 µl)

3.6.4.1 Primers used for mapping in this present study

SSR markers from different sources were used for parental polymorphism survey (Table 3.3). Type of primers used for polymorphism survey and their source is described as under:

Table 3.3: Markers utilized for construction of the interspecific linkage map of Bajra Napier Hybrid

S. No	Type of marker	Marker number	Source
1	SSR	41	Azevedo <i>et al.</i> , 2012
2		56	Kandel <i>et al.</i> , 2016
3		87	Lopez <i>et al.</i> , 2018
4		33	Zhou <i>et al.</i> , 2018
	Total	217	

3.6.5 PCR amplification

3.6.5.1 Parental polymorphism Survey

The SSR primers as shown above (Table 3.3) were used for polymorphism survey. Each primer was tested for polymorphism at different annealing temperatures using DNA from both parents. The polymorphic SSRs were used for genotyping of immortalized F₂ mapping family. For amplification of genomic DNA, a reaction mixture of 10.0 µl volume was prepared using 3.5 µl of sterilized distilled water, 2.0 µl template DNA (13 ng/ µl), 0.5 µl of forward and 0.5 µl of reverse primer (5 µM), 3.5 µl of master mix (Hi Media). The amplifications were carried out in Biometra Advanced 384[®] using PCR protocol as given in Table 3.4.

The amplification products were electrophoresed in 3.0 % agarose gel (depending on the resolution pattern, along with size markers and stained with ethidium bromide having 0.5 µg/ml concentration). Gels were prepared and run in 1X TBE buffer and visualization of fragments was done using Gel Documentation Unit. Sizing of alleles was done with the help of 100-bp DNA ladder.

Table 3.4: PCR conditions used for amplification of BN Hybrid genomic DNA

SSRs and their source	Steps	Temperature	Time(min)
41 SSRs (Azevedo <i>et al.</i> 2012)	Initial denaturation	94	5
	Denaturation	94	1
	Annealing	48-54	1
	Extension	72	2
	Final extension	72	10
	Storage	04	-
56 SSRs (Kendel <i>et al.</i> 2016)	Initial denaturation	94	5
	Denaturation	94	1
	Annealing	50-55	1
	Extension	72	2
	Final extension	72	10
	Storage	04	-
87 SSRs (Lopez <i>et al.</i> 2018)	Initial denaturation	94	5
	Denaturation	94	1
	Annealing	46-58	1
	Extension	72	2
	Final extension	72	10
	Storage	04	-
33 SSRs (Zhou <i>et al.</i> 2018)	Initial denaturation	94	5
	Denaturation	94	1
	Annealing	50-58	1
	Extension	72	2
	Final extension	72	10
	Storage	04	-

3.6.5.2 Validation of polymorphic primers

The markers which were selected from the polymorphic survey were again checked in the population of 14 randomly selected lines. The markers which were polymorphic and showing segregation in the population were selected for final genotyping.

3.6.6 Agarose gel electrophoresis

Three per cent agarose gel containing 0.5 µg/ml ethidium bromide was used to separate PCR amplification products. The gel was prepared according to manufacturer's instructions. Briefly, for 3.0 % agarose gel, agarose was added to 1X TBE buffer. Care was taken to avoid the formation of agarose clumps in the buffer and mixed well. After the addition, the solution was boiled in microwave for 4-5 min. The conical flask was swirled in order to dissolve the agarose properly. After completion, the fully dissolved solution was cooled down to 55°C, and gel was cast after adding the EtBr (0.15µg/ml). The PCR products loaded on gel and electrophoresed at 6V/cm for 2 h in 1X TBE.

3.7 Genotypic analysis

All the genomic SSR primers developed in this study were utilized for the polymorphism analysis between the parents of the mapping population *i.e.*, *C. americanus* and *C. purpureus*. The markers which exhibited polymorphism were selected for genotyping of immortalized F₂ mapping population. The PCR amplification was carried out using the protocol and conditions mentioned in Table 3.4. The PCR products were electrophoresed on 3 per cent agarose gel along with size markers and stained with ethidium bromide. The gels were analyzed in gel documentation unit (IN GENIUS, Syngene Bio Imaging, UK).

3.7.1 Generation of data

The amplified banding patterns were scored manually as 'A' for *C. americanus* type banding pattern, 'B' for *C. purpureus* type banding pattern and H for heterozygous loci. The data matrix was used as an input files for map construction using JOINMAP® 4.1 program (van Ooijen, 2006).

3.7.2 Linkage analysis and map construction

The chi square test was performed for identification of markers with aberrant segregation using the locus genotyping frequencies of JOINMAP. To identify linkage groups, grouping of markers were performed using the minimum independence LOD threshold of 3 and a maximum of 8.0 with a step up of 0.5. The groups showing maximum number of markers and highest linkage at the variable LODs were selected. In this study at LOD 4.0 the groups were converted to maps with the help of the regression algorithm with

the following settings: used linkages with REC smaller than: 0.49, LOD larger than: 0.01, threshold for removal of loci with respect to jumps in goodness-of-fit: 5.0, number of added loci after which to perform a ripple: 2. Distance was calculated using Kosambi's mapping function (Kosambi, 1994).

3.7.3 QTL mapping

3.7.3.1 Phenotypic data evaluation

The phenotypic data for the population, *C. americanus* and *C. purpureus* was obtained as shown above. Statistical analysis of the data, such as ANOVA, frequency distribution, variation coefficient and correlation coefficient analysis, was done using R package version 0.1.6.9000. The phenotypic correlations between each pair of traits were obtained using the Pearson's correlations coefficient applied on the individual phenotypic values. These correlations were tested assuming global significance level of 0.05, 0.01 and 0.001.

3.7.3.2 Statistical analysis for QTL mapping

Quantitative trait loci analysis was carried out on the set of 250 immortalized F₂ individuals with phenotypic data for biomass related traits. The genotypic data consisted of 100 marker loci. QTLs were detected with the Windows QTL Cartographer V2.5 software (Wang *et al.*, 2005) by composite interval mapping (CIM) method (Zeng, 1993, 1994) using the Zmapqtl standard model 6 with a window size of 10 cM and a 2 cM walk speed. The forward regression algorithm was used to obtain cofactors. A 1000-permutation test of shuffling the phenotypes means with the genotypes was performed to estimate a genome-wide LOD score threshold for a QTL at a significance level of P=0.05 (Doerge and Churchill, 1996). An LOD threshold score of >3.0 at 1000 permutations were significantly considered (5% level of significance) to identify and to map the QTLs on the BN hybrid LGs. The 95% confidence intervals of the QTL locations were determined by one-LOD intervals surrounding the QTL peak (Mangin *et al.*, 1994). The estimated additive effect and the percentage of phenotypic variation explained by each putative QTL were obtained using the software with the CIM model by the Zmapqtl procedure. The R² value from this analysis was accepted as the percent phenotypic variance explained by the locus.

The present investigation entitled “**Construction of framework linkage map and genetic dissection of biomass related traits in Bajra-Napier Hybrid**” was carried out to construct first ever framework linkage map of Bajra Napier Hybrid using SSR markers and to identify quantitative traits loci linked to biomass related traits.

The results obtained on different aspects of the present study have been presented and discussed below under following heads:

- 4.1 Linkage map construction using PCR-based markers**
 - 4.1.1 Genotyping of mapping population**
 - 4.1.2 Construction of an interspecific linkage map of Bajra Napier Hybrid**
- 4.2 Analysis of quantitative trait loci**
 - 4.2.1 Analysis of morphological traits**
 - 4.2.1.1 Analysis of variance and trait correlations**
 - 4.2.1.2 Mean performance**
 - 4.2.1.3 Genetic variability parameters**
 - 4.2.1.4 Heritability and genetic advance over mean**
 - 4.2.1.5 Correlation coefficient analysis**
 - 4.2.1.6 Principal Component Analysis and Biplot analysis**
 - 4.2.1.7 Cluster Analysis**
- 4.3 QTLs for biomass related traits in Bajra Napier Hybrid**
 - 4.3.1 Trait dissection**
 - 4.3.1.1 Morphological traits**
 - 4.3.1.2 Phenological traits**
 - 4.3.1.3 Physiological traits**
 - 4.3.1.4 Yield and related traits**
- 4.4 Future research prospects**

4.1 Linkage map construction using PCR-based markers

4.1.1 Genotyping of mapping population

A total of 217 SSRs from different sources were used for parental polymorphism survey (Azevedo *et al.*, 2012; Kendel *et al.*, 2016; Lopez *et al.*, 2018; Zhou *et al.*, 2018).

Out of these, 138 SSRs were found polymorphic for both the parents. The microsatellite markers identified in this study were highly polymorphic. These polymorphic primers were again validated on set of 14 randomly selected F₂ lines along with 2 parents. Only those primers which showed segregation with respect to the parental bands were selected for further genotyping of whole F₂ population. Out of 138 polymorphic SSRs, 100 were selected for further genotyping studies. Of all the 100 SSR markers, 88 (88%) were mapped on seven linkage groups, whereas twelve of the markers were found unlinked. The amplicons generated using SSR primers have been shown in Fig. 4.1. The summary of total markers used along with per cent polymorphism is presented in Table 4.1.

Table 4.1: Microsatellite markers used for construction of inter-specific linkage map of Bajra Napier Hybrid

S. No.	Details of SSR markers	Total Primers	Polymorphic Markers	Percent Polymorphism	Markers Validated	Markers Mapped
1	Azevedo <i>et al.</i> (2012)	41	27	65.8	17	14
2	Kendel <i>et al.</i> (2016)	56	41	73.2	25	22
3	Lopez <i>et al.</i> (2018)	87	51	58.6	37	33
4	Zhou <i>et al.</i> (2018)	33	19	57.5	21	19
	Total	217	138	63.5	100	88

4.1.2 Construction of an interspecific linkage map of Bajra Napier Hybrid

A framework linkage map of Bajra Napier Hybrid was constructed using the genotyping data of 100 polymorphic markers using JoinMap software, version 4.1 (van Ooijen, 2006). A total of 88 SSR markers were assigned positions on seven linkage groups (LGs) at LOD 4.0, based on the number of chromosomes of Bajra Napier Hybrid. The generated linkage map of Bajra Napier Hybrid using these co-dominant markers spanned 858.08 cM distance with an average marker density of 9.75 cM (Fig. 4.2). Of the 88 mapped SSR markers, 14 were from Azevedo *et al.*, 2012; 22 from Kendel *et al.*, 2016, 33 from Lopez *et al.*, 2018 and 19 from Zhou *et al.*, 2018. All the above SSR markers were distributed across seven linkage groups and have been shown in Fig. 4.2. LG1-LG7 were numbered randomly as per JoinMap software.

The details of number of markers mapped in a linkage group, region they spanned and the average marker density exhibited by them have been summarized in Table 4.2. The

length of LGs varied from 20.32 cM in LG3 to 246.43 cM in LG1. The average marker density varied from 5.02 to 34.06 cM, with an average of 9.75 cM, indicating differing degrees of saturation of LGs. The average marker density on each linkage group revealed that the markers were randomly distributed. As the maximum number of markers was mapped in LG1, which harboured 49 markers, the average density was highest in LG1 (5.02 cM) (Fig. 4.2).

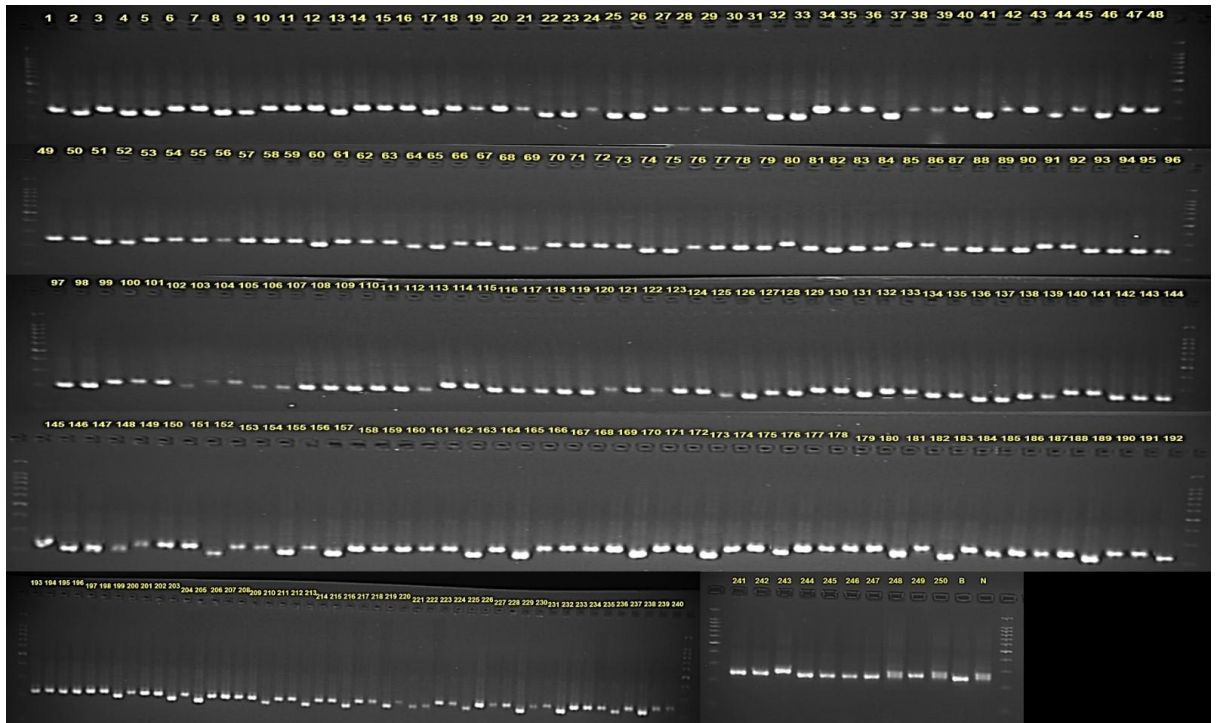


Fig 4.1 SSR banding profile using ICMP 3095 primer on P1 (Bajra), P2 (Napier) and 250 immortalized F₂ lines

Among the 7 linkage groups, each group differed from one another with respect to length and marker distribution. There were 1 large group (LG1), 1 medium (LG2), 4 small groups (LG4, 5, 6 and 7) and one smallest group LG3 which comprised of only 2 markers. As a result of random distribution of markers in the present study, some groups were densely packed (LG1 and LG2), whereas LG3 contained only 2 markers, which can be explained by the fact that SSRs are ubiquitously and randomly distributed in the plant genomes (Ramsay *et al.* 1999; Elsie and Williams, 2001). Most of the markers were located on the centromeric region due to the lower recombination in these regions (Areshchenkova and Ganai 1999; Ramsay *et al.* 2000). The genomic origin of DNA

sequences used for the SSR identification is also responsible for their unequal distribution on the groups and thus lead to less genome coverage (Tanksley *et al.* 1992).

The largest gap of 66.37 cM was observed on LG4 followed by 57.56 on LG5 (Fig. 4.2). This may be due to the occurrence of fewer marker polymorphisms in these gaps or regions thus resulting in lower marker density. Further, this study includes only 88 marker loci to cover the entire genome of BN hybrid covering 7 linkage groups, the number of markers is not sufficient to cover the entire genome of Bajra Napier Hybrid. As the physical size of the bajra genome was estimated to be 1.79 Gbp (Varshney *et al.*, 2017) whereas Napier genome was 1.97 Gbp (Yan *et al.*, 2021), the resultant Bajra Napier hybrid obtained from their cross may have the genome size of 1.93 Gbp. Therefore, more number of markers are required to saturate the map. The similarity between the A and A' genome also reduces the number of polymorphic loci to distinguish the parents. As homozygous regions possess lower recombination frequency and thus may be a possible explanation of low density of markers in these distal regions (Castiglioni *et al.* 1999; Souza *et al.* 2013). Distribution of markers along with their marker density were mentioned in the Table 4.2.

Table 4.2 Distribution of 88 SSR markers on seven linkage groups of an inter-specific linkage map of Bajra Napier Hybrid (*Cenchrus americanus* x *Cenchrus purpureus*)

LGs	Markers mapped	Map length (cM)	Average marker density (cM)
LG1	49	246.43	5.02
LG2	21	176.72	8.41
LG3	2	20.32	10.16
LG4	3	94.1	31.36
LG5	3	86.37	28.79
LG6	3	102.2	34.06
LG7	7	131.9	18.84
Total	88	858.08	9.75

The maximum and minimum distance between markers was 66.37 cM (LG4) and 0.041 cM (LG1), respectively. The distribution of markers between linkage groups was unequal. There was one large group with a stretch of 246.43 cM holding 49 markers, one medium group with a span of 176.72 cM bearing 21 markers and five small groups with length varying from 20.32 to 131.9 cM containing markers in the range of 2-7. The distance between markers on the current map also varied greatly across different linkage

groups, and the size of the LG did not necessarily reflect the number of linked markers. For instance, LG2 with 21 markers covered 176.72 cM with an average marker spacing of 8.41, whereas LG7 spanning a distance of 131.9 cM was covered by 7 markers with average spacing of 18.84 cM.

A great variation in marker densities was observed as many markers showed pronounced clustering. For instance, SSRs of Azevedo *et al.* (2012) were distributed between LG1 and LG2 only. SSRs from Kendel *et al.* (2016) were mapped in each and every linkage group *i.e.*, from LG1 to LG7. SSRs from Lopez *et al.* (2018) also mapped on majority of the genome *i.e.*, from LG1 to LG7 except on LG3. Further, distribution of markers on all the linkage groups were mentioned in the Table 4.3.

Table 4.3: Distribution of various markers among the linkage groups based on marker source

S. No.	Marker source	Linkage group
1	Azevedo <i>et al.</i> (2012)	LG1(10), LG2(04)
2	Kendel <i>et al.</i> (2016)	LG1(13), LG2(03), LG3(01), LG4(01), LG5(01), LG6(01), LG7(02)
3	Lopez <i>et al.</i> (2018)	LG1(14), LG2(11), LG4(01), LG5(01), LG6(01), LG (05)
4	Zhou <i>et al.</i> (2018)	LG1(12), LG2(03), LG3(01), LG4(1), LG5(1), LG6(1)
Total		88

The differences in the crossing-over frequency can influence marker density in a linkage group. Tanksley *et al.*, (1992) explained the uneven marker distribution with the reasoning that centromeres and centromeric heterochromatin and in some instances telomeres experience up to ten fold less recombination. Heterogeneity in recombination along the genome has implications on the development of high resolution linkage maps as the later are much easier to develop for regions of higher recombination. On the other hand, mapping of recombination suppressed regions requires much larger progeny sizes in order to allow the rare recombination events to occur, which is necessary for the construction of fine maps.

There is a large degree of difference with respect to type of mapping population used when compared with previous parental maps. This map was constructed with the help of immortalized F₂ population, but different types of mapping population was used to construct the linkage map in Bajra and Napier (F₂ by Liu *et al.*, 1994; F₂ by Qi *et al.*, 2004; RILs by Pedraza-Garcia *et al.*, 2010; RILs by Supriya *et al.*, 2011; F₂ by Sehgal *et al.*,

2012; F₂ by Rajaram *et al.*, 2013; F_{2:3} by Vengadessan *et al.*, 2013; F₂ by Moumouni *et al.*, 2015; RILs by Punnuri *et al.*, 2016; RILs by Durgaraju *et al.*, 2017; RILs by Saritha *et al.*, 2017; F₂ by Maganlal *et al.*, 2018 and F₁ by Paudel *et al.*, 2018).

Previous pearl millet and Napier maps (Liu *et al.*, 1994; Qi *et al.*, 2004; Pedraza-Garcia *et al.*, 2010; Supriya *et al.*, 2011; Sehgal *et al.*, 2012; Rajaram *et al.*, 2013; Vengadessan *et al.*, 2013; Moumouni *et al.*, 2015; Punnuri *et al.*, 2016; Durgaraju *et al.*, 2017; Saritha *et al.*, 2017; Maganlal *et al.*, 2018 and Paudel *et al.*, 2018) differed with respect to marker type and number, genome coverage and marker density when compared with the present linkage map. The present map covered 858.08 cM of the Bajra Napier Hybrid genome, which was at variance with those reported in previous parental maps (303 cM in Liu *et al.*, 1994; 472.6 cM in Qi *et al.*, 2004; 1,796 cM in Pedraza-Garcia *et al.*, 2010; 1,148 cM in Supriya *et al.*, 2011; 815.3 cM in Sehgal *et al.*, 2012; 615 cM in Rajaram *et al.*, 2013; 1,019 cM in Vengadessan *et al.*, 2013; 640 cM in Moumouni *et al.*, 2015; 716.7cM in Punnuri *et al.*, 2016; 725.5 cM in Durgaraju *et al.*, 2017; 536.8 cM in Saritha *et al.*, 2017; 248.37 cM in Maganlal *et al.*, 2018 and 1,410 cM in Paudel *et al.*, 2018). The mean marker distance of this map is 9.75 cM, which was at variance with those reported in the previous parental maps (2 cM in Liu *et al.*, 1994; 1.9 cM in Qi *et al.*, 2004; 9.2 cM in Pedraza-Garcia *et al.*, 2010; 0.28 cM in Supriya *et al.*, 2011; 6.13 cM in Sehgal *et al.*, 2012; 5.9 cM Rajaram *et al.*, 2013; 23 cM in Vengadessan *et al.*, 2013; 0.51 cM in Moumouni *et al.*, 2015; 23.23 cM in Punnuri *et al.*, 2016; 9.8 cM in Durgaraju *et al.*, 2017; 13.7 cM in Saritha *et al.*, 2017; 9.19 cM in Maganlal *et al.*, 2018 and 0.73 cM in Paudel *et al.*, 2018).

Several factors including population size and the number of markers used in the analysis may contribute to differences in map coverage (Lauco *et al.*, 1998). Differences in the genetic background of mapping populations could account for some marker rearrangements resulting in variation of size and number of linkage groups in the current and previous genetic maps of parents. According to Paran *et al.*, 1995, the level of stringency (LOD score and recombination frequency), number of markers, and size of mapping populations also have effects on the size and number of linkage groups.

Segregation distortion is a common feature which is observed in most of the linkage maps in plants and has been attributed to difference in DNA content, structural rearrangement, self-incompatibility and deleterious recessive alleles (Berry *et al.*, 1995;

Barzen *et al.*, 1995; Quillet *et al.*, 1995; Jenczewski *et al.*, 1997). In the present study, significantly high distortion was exhibited by 12 % (12/100) of the markers. Thus, it may be inferred that population type is not the only factor which is responsible for distortion but other factors such as chromosome loss during the process of crossing over (Kasha and Kao, 1970), isolation mechanisms (Zamir and Tadmor, 1986) or the presence of other alien or viability genes (Hendrick and Muona, 1990; Bradshaw and Stettler, 1994) may also affect the level of skewed segregation. Segregation distortion in several crosses can be explained by small chromosomal rearrangements with little impact on fertility and chromosome pairing during meiosis (Slocum *et al.*, 1990), linkage to an incompatibility locus (Wricke and Wehling, 1985), or linkage to a lethal allele in gametes (Pillen *et al.*, 1992; Wagner *et al.*, 1992). A clustering of distorted loci has often been reported within the linkage groups constructed in several species (Graner *et al.*, 1991; Kammholz *et al.*, 2001).

4.2 Analysis of quantitative trait loci

Quantitative trait locus (QTL) mapping is a genome-wide inference of the relationship between genotype at various genomic locations and phenotype for a set of quantitative traits in terms of the number, genomic positions, effects and interaction of QTL. The primary purpose of QTL mapping is to localize chromosomal regions that significantly affect the variation of quantitative traits in a population. This localization is important for the ultimate identification of responsible genes and also for our understanding of genetic mechanisms of the variation.

The availability of genetic maps allows the localization and mapping of different agronomically important traits with the help of phenotypic data of segregating populations and in the identification of markers closely linked to the particular trait for marker-assisted selection and positional cloning. Mapping QTL can also help us to understand how many QTL significantly contribute to the trait variation in a population. How much variation is due to the additive effects of QTL and how much is due to dominant and epistatic effects of QTL? What is the nature of genetic correlation between different traits in a genomic region, pleiotropy, or close linkage? Do QTL interact with environments? These questions are related to the genetic architecture of quantitative traits in the population, and are

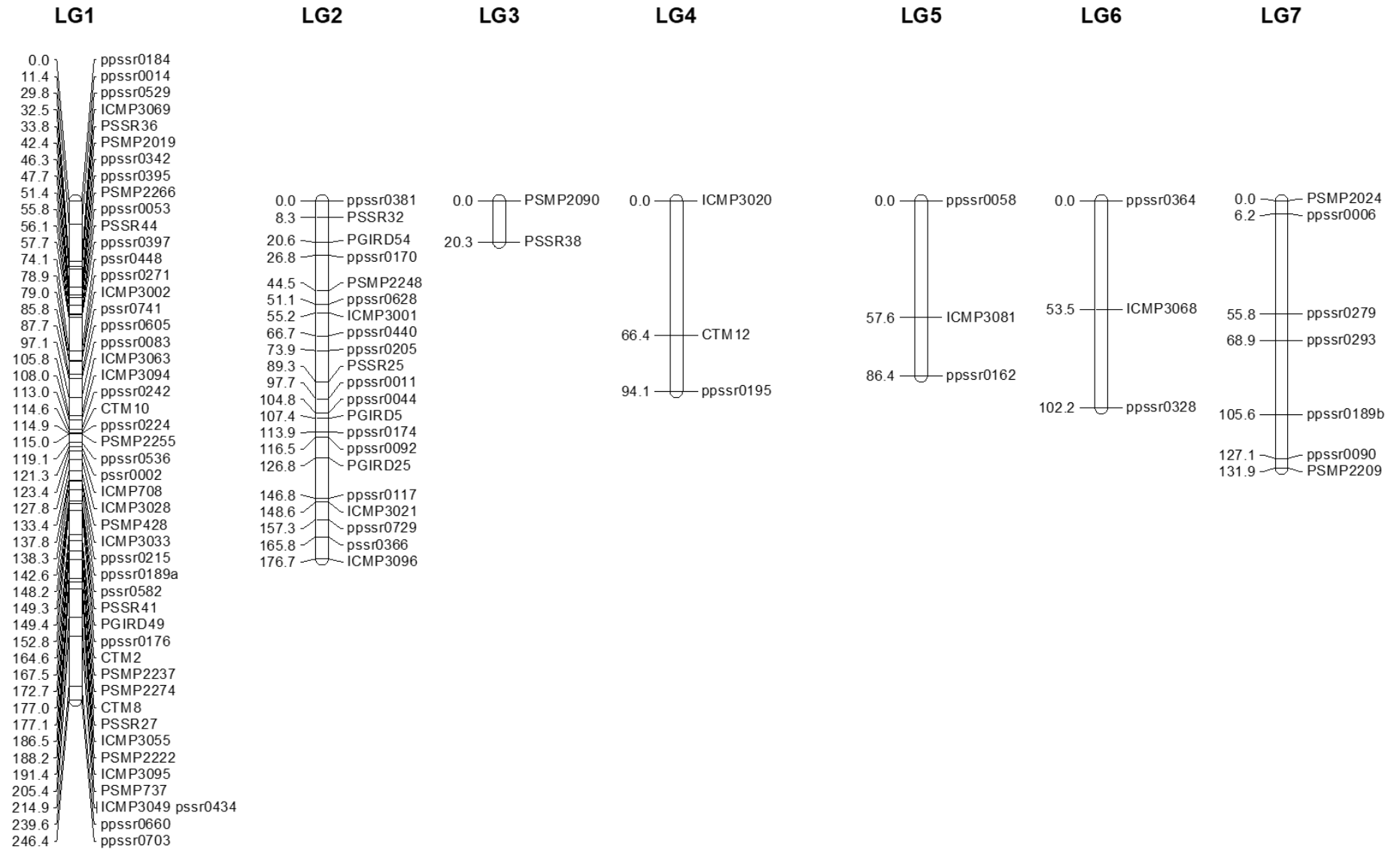


Fig 4.2. An interspecific linkage map of Bajra Napier Hybrid based on immortalized F₂ mapping population generated by crossing *C. americanus* and *C. purpureus*. The map was generated with 88 polymorphic SSR markers using JoinMap version 4.0 at a LOD value of 4.0, with Kosambi mapping function. A total of 88 markers were mapped on seven linkage groups (LG1 to LG7).

intimately related to many applications in quantitative genetics, such as marker-assisted prediction or selection and marker-assisted gene introgression (Zeng, 2001). An effort has been made during the present study to find out solutions for all these questions by constructing the first-ever framework linkage map of Bajra Napier Hybrid followed by mapping of QTLs responsible for biomass related traits in Bajra Napier hybrid. Tightly linked markers with trait of interest will be used for marker assisted breeding (MAB).

Marker-selected selection (MAS) is an important part of MAB which is being used to speed up breeding programs for crop improvement. This is because it is based on relating the phenotyping data with genotyping of DNA marker (Mehta *et al.* 2019). Molecular breeding involves the use of molecular markers in combination with linkage maps and genomics to improve a particular trait. Various molecular breeding strategies include marker-assisted selection (MAS), marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS), and genomic selection or genomic predictions (GS) (Ribaut *et al.*, 2010). Marker-assisted selection involves indirect phenotypic selection in which individuals are selected based on the marker pattern.

Therefore, the present study was undertaken to develop a first ever frame work linkage map of Bajra Napier Hybrid using interspecific immortalized F_2 mapping population using genomic as well as genic SSR markers and to identify the genomic regions associated with biomass related traits. The identification of QTLs controlling agronomically important traits would enable to analyse association between the mapped loci and traits and provide the basis for Bajra Napier Hybrid genomics and breeding.

4.2.1 Analysis of morphological traits

4.2.1.1 Analysis of variance and trait correlations

Significant differences for all the 22 measured traits were observed among the mapping population (Table 4.4). The Analysis of Variance (ANOVA) of 250 immortalized F_2 lines revealed significant differences for all the traits. In general, the mean values of F_2 lines were intermediate to that of parents. The values of F_2 lines outside the parental range were also observed for the traits under study. This indicated that the alleles that increased phenotypic values were dispersed in both parental lines, even when their values differed markedly, thus indicating transgressive segregation for all traits (Fig 4.3). The traits associated with biomass assessed in this study appeared to be quantitatively inherited as

shown by the presence of nearly continuous distribution of mean phenotypic values of the traits for 250 immortalised F_2 lines (Annexure-I).

The ANOVA revealed the presence of non-significant differences between the blocks based on the parameters evaluated indicates that all the traits exhibiting variation. The variation among the treatments showed significant differences which resulted into exploration and selection of suitable breeding lines.

4.2.1.2 Mean performance

Mean values and ranges for all the biomass related traits are shown in Table 4.5 and mean values for each genotype for all characters are given in Appendix-I.

Days to Flowering:

Mean for days to 50 percent flowering was 94.60 days which ranged from 77.37-112.19 days. Whereas, the maximum days to 50% flowering was recorded for F_2 -229, F_2 -249 & F_2 -248 (108 days) and minimum days to 50% flowering was recorded for F_2 -14 & F_2 -23 (81 days). The population showed near normal distribution for this trait as shown in Fig 4.3 (a). The Bajra parent was early flowering (<80 days) whereas Napier was late flowering (100-115 days). However, 50 per cent of the population showed early flowering (85-95 days), 30 per cent of population flowers after 90-100 days and 20 % of the population showed late flowering (101-110 days).

Plant Height:

Mean for plant height was 184.34 cm which ranged from 112.81-287.51 cm. whereas, the maximum plant height was recorded for F_2 -34 (285.2 cm) and minimum plant height was recorded for F_2 -227 (114 cm). The population showed perfect normal distribution for the trait Plant Height as shown in Fig 4.3 (b). The Bajra parent was short (130 cm) whereas Napier was tall (250 cm). Overall, 25% lines were small, 50% were medium and 25% were tall.

Leaf Length:

Mean for leaf length was 40.81 cm which ranged from 22.06-66.03 cm. whereas, the maximum leaf length was recorded for F_2 -1 (62.66 cm) and minimum leaf length was recorded for F_2 -238 (21.68 cm). The Bajra parent was having medium sized leaves (48 cm) whereas Napier was having long size leaves (55 cm). Here we have observed the presence

of transgressive segregants in the population as shown in Fig 4.3 (c). Overall, 30% lines were small, 50% were medium and 20% were tall.

Leaf Width:

Mean for leaf width was 1.72 cm which ranged from 0.81-2.95 cm. Whereas, the maximum leaf width was recorded for F₂-112 (2.84 cm) and minimum leaf width was recorded for F₂-249 (0.86 cm). The population showed normal distribution for the trait Leaf Width as shown in Fig 4.3 (d). The Bajra parent had broad leaves (2.6 cm) whereas Napier had medium sized leaves (2.1 cm). Here also we have observed the transgressive segregants in the population. Overall, 60% lines were narrow, 20% were medium and 20% were broad.

Number of Tillers per Plant:

Mean for number of tillers per plant was 7.24 tillers which ranged from 2.83-17.03 tillers. Whereas, the maximum number of tillers per plant was recorded for F₂-1 (17) and minimum number of tillers per plant was recorded for F₂-80 (2.6). The population showed near normal distribution for the trait Number of Tillers per Plant. Bajra parent was having less number of tillers compared to Napier grass. Here the population slightly skewed towards the bajra parent as shown in Fig 4.3 (e). Around 25% of the population was having less number of tillers similar to Bajra whereas 15% was having more number of tillers similar to Napier grass and remaining 60% are intermediate.

Stem thickness:

Mean for stem thickness was 10.51 mm which ranged from 6.47-18.90 mm. whereas, the maximum stem thickness was recorded for F₂-34 (19.15 mm) and minimum stem thickness was recorded for F₂-199 (6.57 mm). The population showed near normal distribution for the trait Stem Thickness as shown in Fig 4.3 (f). Bajra parent was having thick stem whereas Napier was having medium sized stem. Transgressive segregants were also observed for this trait. Overall, 60% lines were having thin stem, 20% were medium and 20% were of broad stem.

Inter Nodal Length:

Mean for inter nodal length was 12.32 cm which ranged from 7.81-20.30 cm. whereas, the maximum inter nodal length was recorded for F₂-1 (19.62 cm) and minimum

inter nodal length was recorded for F₂-201 (8.23). The population showed perfect normal distribution for the trait Inter Nodal Length as shown in Fig 4.3 (g). Bajra parent was having less intermodal length when compared with Napier. The population was having 25% similarity to each Bajra and Napier whereas remaining 50% were intermediate.

Number of Leaves per Plant:

Mean for number of leaves per plant was 8.23 leaves which ranged from 5.23-13.43 leaves. Whereas, the maximum number of leaves per plant was recorded for F₂-34, F₂-185 & F₂-198 (13.4) and minimum number of leaves per plant was recorded for F₂-175 (5.2). The population showed normal distribution for the trait Number of Leaves per Plant as shown in Fig 4.3 (h). Bajra parent was having less number of leaves as compared to Napier. Overall 25% of population showed less number of leaves, 50% showed intermediate and remaining 25% of population are having more number of leaves.

Spike Length:

Mean for spike length was 29.06 cm which ranged from 18.85-38.50 cm. Whereas, the maximum spike length was recorded for F₂-34 & F₂-29 (38.2 cm) and minimum spike length was recorded for F₂-226 (18.8 cm). The population showed normal distribution for the trait Spike Length as shown in Fig 4.3 (i). Bajra parent was having more spike length compared to Napier. Overall 25% of the population bears short spike length, 50% bears intermediate sized one and remaining 25% bears long spikes.

Number of Seed per Plant:

Mean for number of seed per plant was 145.54 seeds which ranged from 11.50-546.00 seeds. Whereas, the maximum number of seed per plant was recorded for F₂-222 (550) and minimum number of seed per plant was recorded for F₂-135 (13). The population showed normal distribution for the trait Number of Seed per Plant as shown in Fig 4.3 (j). Bajra parent produced more number of seed when compared to Napier. In population, 25% of the population produced less number of seed, 25% produced more number of seed and remaining 50% produced the seed in medium amount.

Green Leaf Weight per Plant:

Mean for green leaf weight per plant was 0.27 Kg which ranged from 0.02-1.13 Kg. Whereas, the maximum green leaf weight per plant was recorded for F₂-34 (1.13 Kg) and

Table 4.4 Analysis of variance (ANOVA) for biomass related traits in Bajra Napier Hybrid

ANOVA for treatment adjusted																							
Mean.Sq																							
Source	Df	DTF	PH	LL	LW	NTPP	ST	INL	NLPP	SPL	NSPP	GLW_PP	GSW_PP	LDW_PP	SDW_PP	LSR	DMY_PP	GFY_PP	CHL	Oxalate	SL	SB	TSW
Block (ignoring Treatments)	7	275.51 **	3638.23 **	102.84 **	0.33 **	53.19 **	9.81 **	2.68 **	1.60 **	59.38 **	7741.34 **	0.12 **	0.60 **	0.01 **	0.09 **	0.02 **	0.14 **	17.38 **	54.28 **	1.96 **	0.08 *	0.04 **	2.11 **
Treatment (eliminating Blocks)	251	48.77 **	957.18 **	40.43 *	0.13 **	6.17 **	4.69 **	3.11 **	2.10 **	13.51 **	8201.50 **	0.04 **	0.18 **	0.00 **	0.03 **	0.01 **	0.04 **	4.38 **	33.91 **	0.36 **	0.15 **	0.06 **	1.23 **
Treatment: Check	1	3306.25 **	52001.00 **	264.06 **	0.98 **	222.61 **	32.23 **	105.06 **	59.29 **	231.42 **	574862.25 **	0.11 **	0.62 **	0.01 **	0.00 **	0.15 **	0.03 **	25.00 **	72.97 **	24.95 **	12.06 **	3.05 **	139.59 **
Treatment: Test and Test vs. Check	250	35.74 **	713.01 **	39.54 *	0.13 **	5.30 **	4.58 **	2.70 **	1.87 **	12.64 **	5534.86 **	0.04 **	0.18 **	0.00 **	0.03 **	0.01 **	0.04 **	4.30 **	33.76 **	0.26 **	0.10 **	0.05 **	0.68 **
Residuals	7	0.68	13.71	9.63	0.01	0.00	0.00	0.04	0.01	0.17	18.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.37	0.01	0.02	0.00	0.00
ANOVA for block adjusted																							
Mean.Sq																							
Source	Df	DTF	PH	LL	LW	NTPP	ST	INL	NLPP	SPL	NSPP	GLW_PP	GSW_PP	LDW_PP	SDW_PP	LSR	DMY_PP	GFY_PP	CHL	Oxalate	SL	SB	TSW
Treatment (ignoring Blocks)	251	55.98 **	1058.44 **	43.26 *	0.14 **	7.65 **	4.96 **	3.19 **	2.14 **	15.16 **	8416.31 **	0.04 **	0.19 **	0.00 **	0.03 **	0.01 **	0.05 **	4.86 **	35.40 **	0.42 **	0.15 **	0.06 **	1.29 **
Treatment: Check	1	3306.25 **	52001.00 **	264.06 **	0.98 **	222.61 **	32.23 **	105.06 **	59.29 **	231.42 **	574862.25 **	0.11 **	0.62 **	0.01 **	0.00 **	0.15 **	0.03 **	25.00 **	72.97 **	24.95 **	12.06 **	3.05 **	139.59 **
Treatment: Test	249	42.40 **	810.90 **	36.66 *	0.11 **	6.33 **	3.92 **	2.79 **	1.80 **	14.17 **	5288.22 **	0.04 **	0.19 **	0.00 **	0.03 **	0.01 **	0.05 **	4.13 **	31.84 **	0.32 **	0.09 *	0.05 **	0.55 **
Treatment: Test vs. Check	1	185.79 **	1753.40 **	1466.55 **	6.77 **	121.72 **	236.52 **	0.52 **	31.09 **	44.02 **	120864.38 **	0.07 **	0.23 **	0.01 **	0.01 **	0.27 **	0.00 **	168.28 **	883.87 **	0.77 **	4.29 **	0.28 **	47.54 **
Block (eliminating Treatments)	7	16.96 **	7.25 ns	1.21 ns	0.01 ns	0.01 *	0.00 ns	0.08 ns	0.09 **	0.49 ns	39.14 ns	0.00 ns	0.00 ns	0.00 ns	0.00 ns	0.00 ns	0.00 ns	0.00 ns	0.86 ns	0.00 ns	0.01 ns	0.00 ns	0.01 ns
Residuals	7	0.68	13.71	9.63	0.01	0.00	0.00	0.04	0.01	0.17	18.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.37	0.01	0.02	0.00	0.00
ns P > 0.05; * P <= 0.05; ** P <= 0.01																							

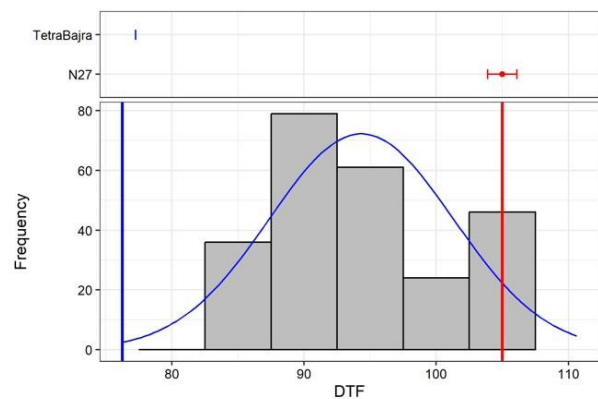


Fig 4.3 (a)

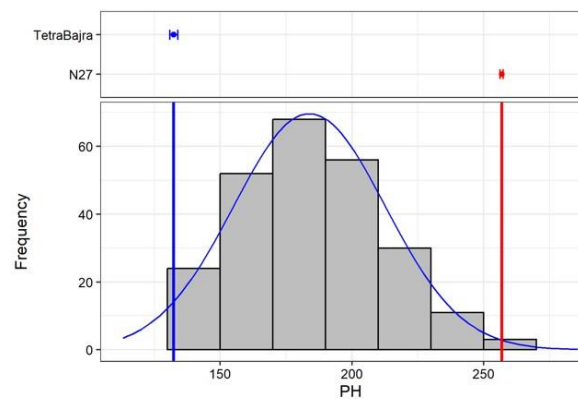


Fig 4.3 (b)

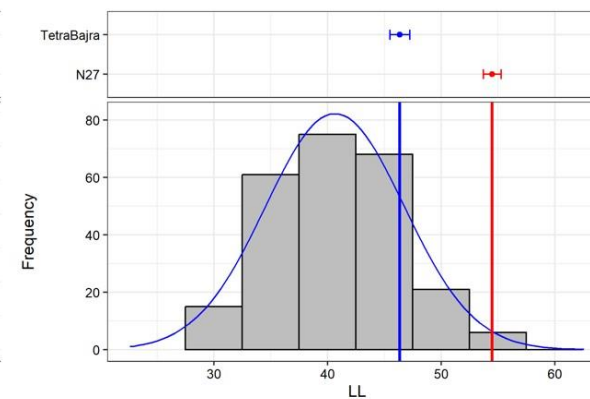


Fig 4.3 (c)

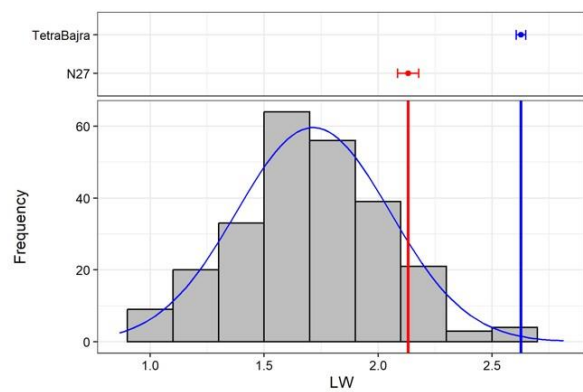


Fig 4.3 (d)

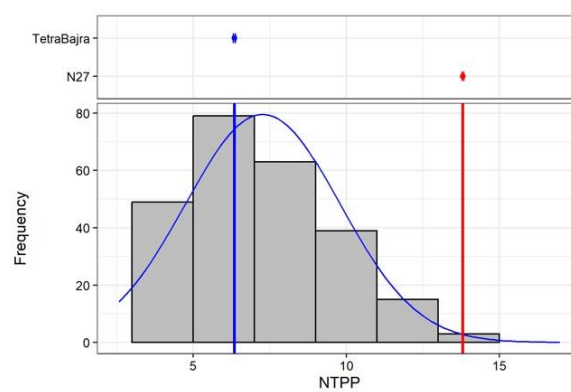


Fig 4.3 (e)

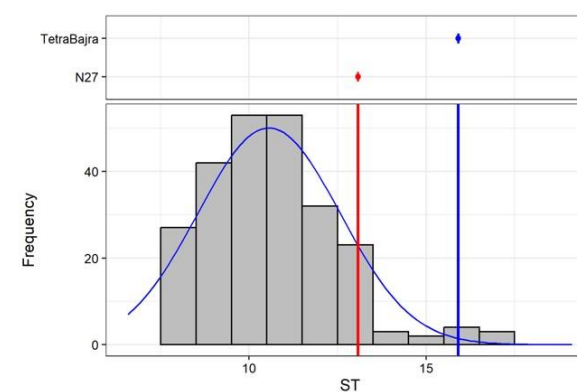


Fig 4.3 (f)

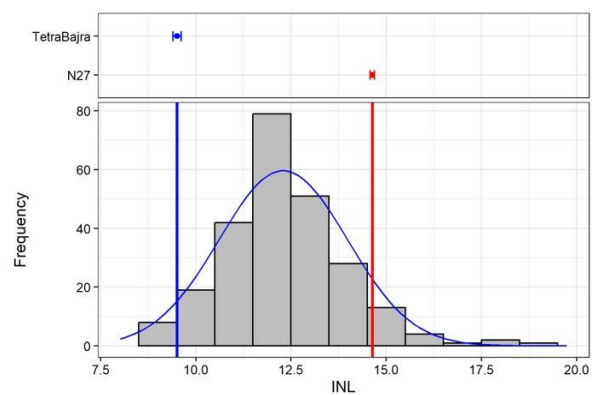


Fig 4.3 (g)

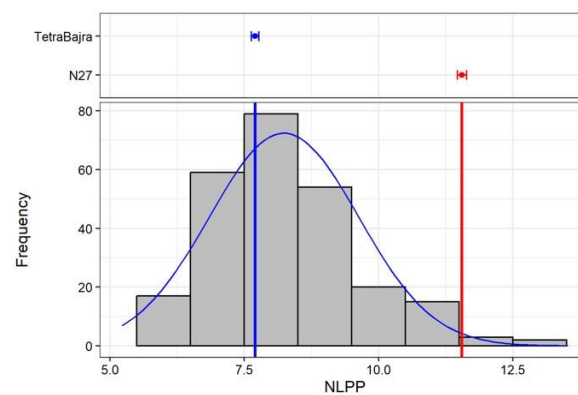


Fig 4.3 (h)

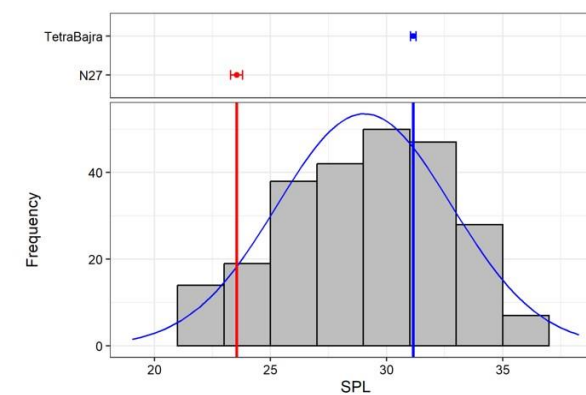


Fig 4.3 (i)

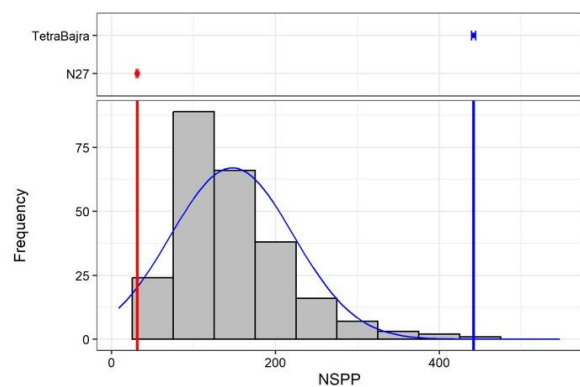


Fig 4.3 (j)

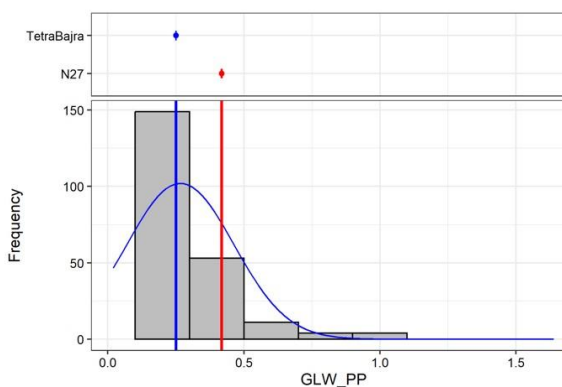


Fig 4.3 (k)

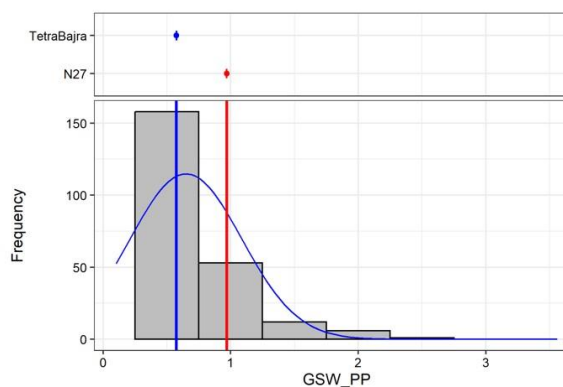


Fig 4.3 (l)

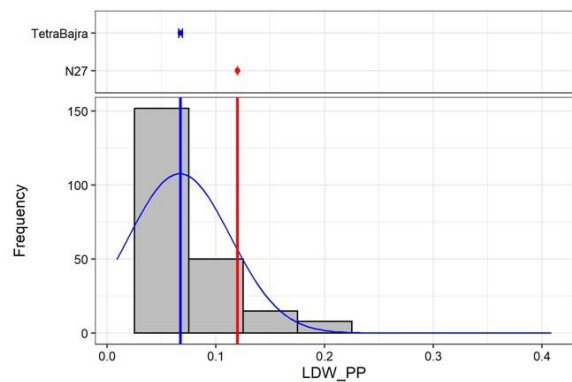


Fig 4.3 (m)

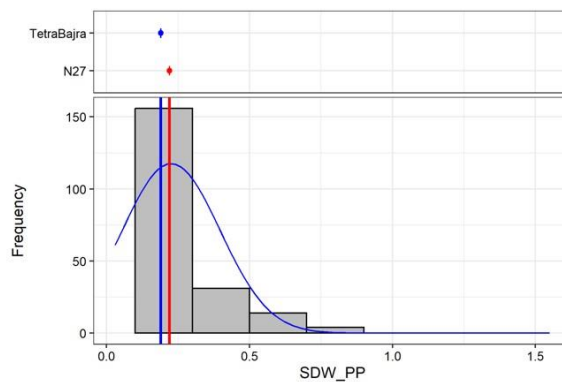


Fig 4.3 (n)

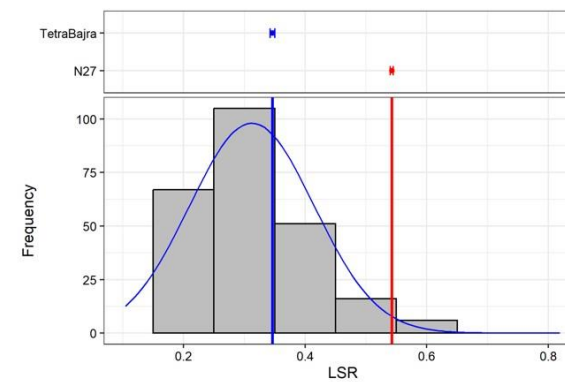


Fig 4.3 (o)

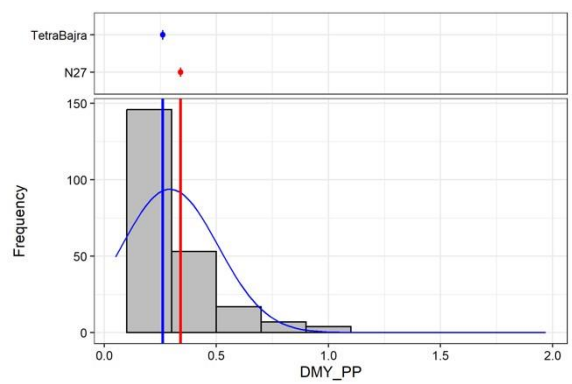


Fig 4.3 (p)

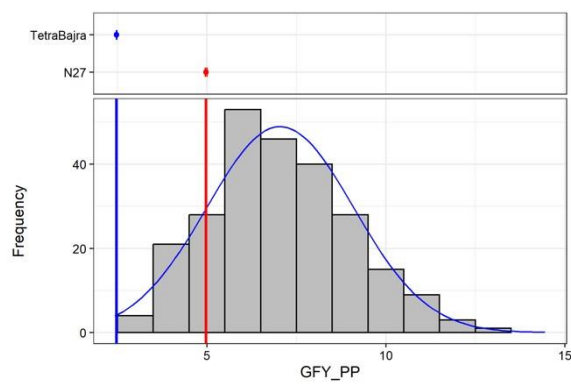


Fig 4.3 (q)

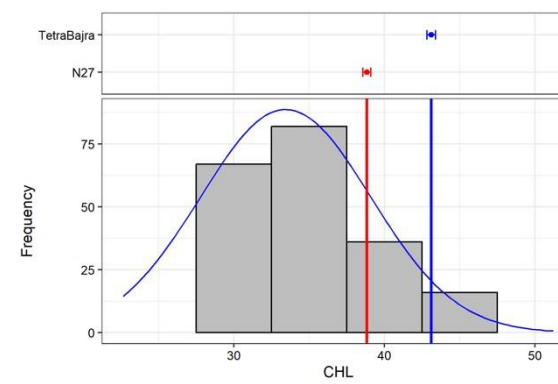


Fig 4.3 (r)

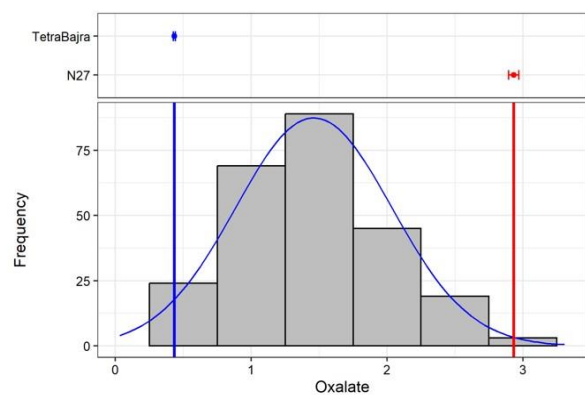


Fig 4.3 (s)

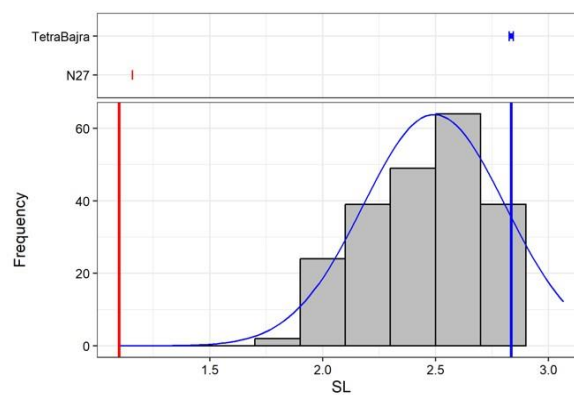


Fig 4.3 (t)

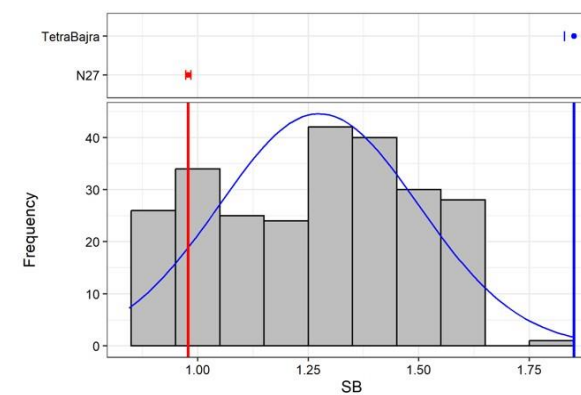


Fig 4.3 (u)

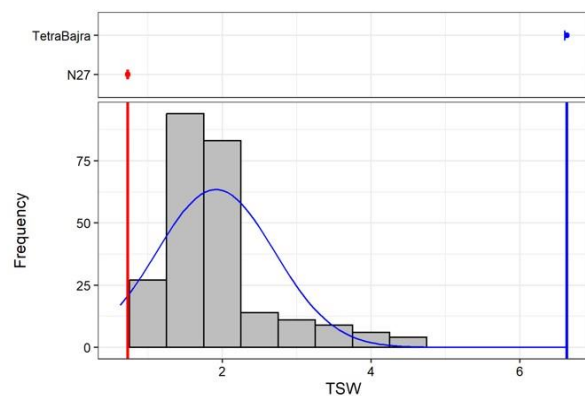


Fig 4.3 (v)

Fig 4.3 Graphical representation of frequency distribution of 250 immortalized F₂ population for 22 biomass related trait

minimum green leaf weight per plant was recorded for F₂-177 (0.02 Kg). The population showed near normal distribution with some skewness towards Bajra parent for the trait Green Leaf Weight per Plant as shown in Fig 4.3 (k). Overall 50% of the population was similar to Bajra while, 30% was similar to Napier and remaining 20% showed transgressive segregation.

Green Stem Weight per Plant:

Mean for green stem weight per plant was 0.65 Kg which ranged from 0.10-2.86 Kg. whereas, the maximum green stem weight per plant was recorded for F₂-34 (2.86 Kg) and minimum green stem weight per plant was recorded for F₂-177 (0.10 Kg). The population showed near normal distribution with some skewness towards Bajra parent for the trait Green Stem Weight per Plant as shown in Fig 4.3 (l). Overall 50% of the population was similar to Bajra and 30% was similar to Napier while, remaining 20% showed transgressive segregation.

Leaf Dry Weight per Plant:

Mean for leaf dry weight per plant was 0.07 Kg which ranged from 0.01-0.29 Kg. whereas, the maximum leaf dry weight per plant was recorded for F₂-34 (0.29 Kg) and minimum leaf dry weight per plant was recorded for F₂-177 (0.01 Kg). The population showed near normal distribution with some skewness towards Bajra parent for the trait Leaf Dry Weight per Plant as shown in Fig 4.3 (m). Overall 50% of the population was similar to Bajra and 30% was similar to Napier while, remaining 20% showed transgressive segregation.

Stem Dry Weight per Plant:

Mean for stem dry weight per plant was 0.22 Kg which ranged from 0.03-0.95 Kg. whereas, the maximum stem dry weight per plant was recorded for F₂-34 (0.95 Kg) and minimum stem dry weight per plant was recorded for F₂-198 (0.03 Kg). The population showed near normal distribution with some skewness towards Bajra parent for the trait Stem Dry Weight per Plant as shown in Fig 4.3 (n). Overall 50% of the population was similar to Bajra and 30% was similar to Napier while, remaining 20% showed transgressive segregation.

Leaf Stem Ratio:

Mean for leaf stem ratio was 0.31 which ranged from 0.11-0.82. Whereas, the maximum leaf stem ratio was recorded for F₂-224 (0.82) and minimum leaf stem ratio was recorded for F₂-11 (0.11). The population showed normal distribution for the trait Leaf to Stem Ratio as shown in Fig 4.3 (o). Napier was having more Leaf to Stem Ratio when compared to Bajra. Over all 25% of population was similar to Napier while 50% was having similarity with Bajra and remaining 25% showed transgressive segregation.

Dry Matter Yield per Plant:

Mean for dry matter yield per plant was 0.29 Kg which ranged from 0.05-1.24 Kg. whereas, the maximum dry matter yield per plant was recorded for F₂-334 (1.24 Kg) and minimum dry matter yield per plant was recorded for F₂-198 (0.05 Kg). The population showed near normal distribution with some skewness towards Bajra parent for the trait Dry Matter Yield per Plant as shown in Fig 4.3 (p). Overall 50% of the population was similar to Bajra and 30% was similar to Napier while, remaining 20% showed transgressive segregation.

Green Fodder Yield per Plant:

Mean for green fodder yield per plant was 7.05 Kg which ranged from 2.41-14.73 Kg. whereas, the maximum green fodder yield per plant was recorded for F₂-61 (14.73 Kg) and minimum green fodder yield per plant was recorded for F₂-180 (2.41 Kg). The population showed perfect normal distribution for the trait Green Leaf Weight per Plant as shown in Fig 4.3 (q). Overall 5% of the population was similar to Bajra and 30% was similar to Napier while, remaining 65% showed transgressive segregation.

Chlorophyll Content:

Mean for chlorophyll content was 33.42 which ranged from 22.71-51.29. Whereas, the maximum chlorophyll content was recorded for F₂-116 (51.29) and minimum chlorophyll content was recorded for F₂-121 (23.6). The population showed near normal distribution for the trait Chlorophyll content as shown in Fig 4.3 (r). Overall 25% of the population was similar to Bajra and 25% was similar to Napier while, remaining 50% showed intermediate expression.

Oxalate content:

Mean for oxalate content was 1.46 % which ranged from 0.04-3.31 %. Whereas, the maximum oxalate content was recorded for F₂-204 (3.31 %) and minimum oxalate content was recorded for F₂-131 (0.1 %). The population showed perfect normal distribution for the trait Oxalate content as shown in Fig 4.3 (s). Overall 25% of the population was similar to Bajra and 25% was similar to Napier while, remaining 50% showed intermediate expression.

Seed Length:

Mean for seed length was 2.49 mm which ranged from 1.11-3.21 mm. Whereas, the maximum seed length was recorded for F₂-31, F₂-41 & F₂-51 (3.1 mm) and minimum seed length was recorded for F₂-61 & F₂-101 (2 mm). The population showed near normal distribution with some skewness towards Bajra parent for the trait Seed length as shown in Fig 4.3 (t). Overall 60% of the population was similar to Bajra while, 35% of the population was showing intermediate expression and very little amount (<5%) of the population showed similarity with the Napier grass.

Seed Breadth:

Mean for seed breadth was 1.28 mm which ranged from 0.82-2.25 mm. Whereas, the maximum seed breadth was recorded for F₂-41 (1.8 mm) and minimum seed breadth was recorded for F₂-233 (0.82 mm). The population showed near normal distribution for the trait Seed breadth as shown in Fig 4.3 (u). Overall 40% of the population was similar to Bajra and 10% was similar to Napier while, remaining 50% showed intermediate expression.

Thousand Seed weight:

Mean for thousand seed weight was 1.97 g which ranged from 0.59-10.31 g. Whereas, the maximum thousand seed weight was recorded for F₂-41 (4.77) and minimum thousand seed weight was recorded for F₂-133 (0.62 g). The population showed near normal distribution with some skewness towards Bajra parent for the trait Thousand Seed Weight as shown in Fig 4.3 (v). Overall 50% of the population was similar to Bajra, nearly 50% of population showed medium range and very little showed similarity with Napier grass.



Fig 4.4 Field view of immortalized F₂ lines of Bajra Napier Hybrid



Fig 4.5 Field view of immortalized F₂ lines of Bajra Napier Hybrid (after 1st cut)



Fig 4.6 Field view of immortalized F₂ lines of Bajra Napier Hybrid (after 3rd cut)



Fig 4.7 Some promising F₂ lines of Bajra Napier Hybrid from the population

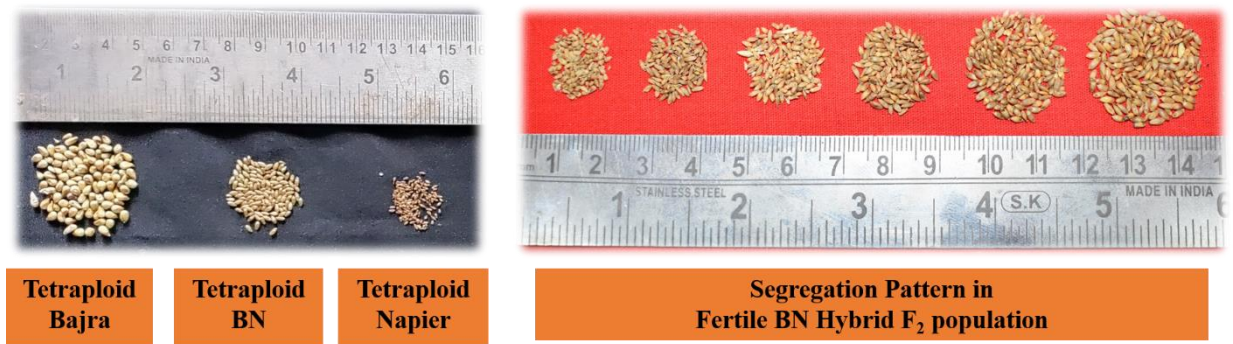


Fig 4.8 Variation of seed size in immortalized F₂ population along with their parents



Fig 4.9 (a) Transgressive segregants for the trait stem thickness



Fig 4.9 (b) Transgressive segregants for the trait spike length



Fig 4.9 (c) Transgressive segregants for the trait Leaf length and leaf width

Near-normal distributions were observed for flowering date, plant height, and number of tillers, stem diameter and leaf width confirming the quantitative nature of these traits. Similar distribution for these traits were observed by Sinche *et al.*, 2021 in F₁ mapping population.

4.2.1.3 Genetic variability parameters

Existence of sufficient variability in plant genetic resources (PGR) provides opportunity for plant breeders to develop improved cultivars with desired characters and improvement through selection depends on level of genetic variability exist. The 250 immortalised F₂ lines were analysed for 22 traits to estimate variability parameters like genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), genetic advance as percent of mean and heritability (broad sense) and estimates for these parameters are presented in Table 4.6.

Genotypic and phenotypic coefficient of variation:

In order to evaluate genetic variation and determine the degree of overall variance, the coefficient of variation was estimated using the outcomes of analysis of variance for all of the examined characteristics. The significant differences between GCV and PCV demonstrate that the certainty is due to the favourable influence of the environment as well as the genotypes' genetic composition.

Highest GCV was observed in the trait Stem Dry Weight per Plant (76.04) followed by Green leaf Weight per Plant (74.25), Dry matter Yield per Plant (73.57), Leaf Dry Weight Per Plant (70.18), Green Stem Weight per Plant (67.62), Number of Seed per Plant (49.40), Thousand Seed Weight (38.77), Oxalic acid percent (38.15), Number of Tillers per Plant (34.63), Leaf to Stem Ratio (32.49), Green Fodder Yield per Plant (28.83), Stem Thickness (18.75), Leaf Width (18.06), Seed Breadth (17.16), Chlorophyll content (16.78), Number of Leaves per Plant (16.23), Plant Height (15.36), Inter Nodal length (13.48), Spike Length (12.89), Leaf Length (12.79), Seed length (10.73) and Days to 50% flowering (6.85).

High PCV estimate was found in the Seed Dry Weight per Plant (76.04) followed by Green Leaf Weight (74.26), Dry Matter Yield per Plant (73.57), Leaf Dry Weight per Plant (70.35), Green Stem Weight per Plant (67.72), Number of Seed per Plant (49.49), Thousand Seed Weight (38.84), Oxalic acid per cent (38.57), Number of Tillers per Plant

(34.63), Leaf Stem Ratio (32.71), Green Fodder yield per Plant (28.84), Leaf Width (19.37), Stem Thickness (18.76), Seed Breadth (17.40), Chlorophyll content (16.88), Number of Leaves per Plant (16.27), Plant Height (15.49), Leaf Length (14.89), Inter Nodal Length (13.58), Spike Length (12.96), Seed Length (11.83) and Days to 50% Flowering (6.90).

In the current study, most of the characters evaluated had greater PCV than GCV (Fig 4.10 (a)). The traits like Stem Dry Weight per Plant and Dry Matter Yield per Plant have absolutely no effect of environment on trait expression. Subsequently, the traits like Leaf Length followed by Leaf Width and Seed Width have significant effect of environment.

Table 4.5: Range, mean and standard deviation for biomass related traits in Bajra Napier Hybrid

Trait	Mean	Std. Deviation	Range
DTF	94.6	7.77	77.37-112.19
PH	184.34	29.76	112.81-287.51
LL	40.81	6.51	22.06-66.03
LW	1.72	0.35	0.81-2.95
NTPP	7.24	2.53	2.83-17.03
ST	10.51	1.99	6.47-18.90
INL	12.32	1.73	7.81-20.30
NLPP	8.23	1.37	5.23-13.43
SPL	29.06	3.84	18.85-38.50
NSPP	145.54	73.72	11.50-546.00
GLW_PP	0.27	0.19	0.02-1.13
GSW_PP	0.65	0.42	0.10-2.86
LDW_PP	0.07	0.04	0.01-0.29
SDW_PP	0.22	0.16	0.03-0.95
LSR	0.31	0.1	0.11-0.82
DMY_PP	0.29	0.2	0.05-1.24
GFY_PP	7.05	2.06	2.41-14.73
CHL	33.42	5.65	22.71-51.29
Oxalate	1.46	0.57	0.04-3.31
SL	2.49	0.32	1.11-3.21
SB	1.28	0.24	0.82-2.25
TSW	1.97	0.92	0.59-10.31

4.2.1.4 Heritability and genetic advance over mean

A character's genetic makeup and the manner in which genetic variation is passed between genotypes are both considered to be heritable traits, which are important aspects of genetics. It shows genotype breeding values for particular traits, which are percentages of the overall variance passed down from parents to offspring.

Heritability and genetic advance (GA) are important biometric techniques for analysing population variation with the aim of selecting and assessing acceptable germplasm for breeding procedures. Under selection pressures, genetic advance is defined as a shift in allele frequency towards the superior direction, and it is often stated as a percentage of the mean (genetic gain). Genetic advance measures the degree of variation that responds to selection. Because genetic variance is important in selection, it demonstrates additive variance, which quantifies additive gene expression and is crucial for genetic gain under selection.

Table 4.6: Parameters of genetic variability for biomass related traits in Bajra Napier Hybrid

Trait	Mean	GCV	GCV category	PCV	PCV category	hBS	hBS category	GAM	GAM category
DTF	94.32	6.85	Low	6.90	Low	98.40	High	14.01	Medium
PH	183.83	15.36	Medium	15.49	Medium	98.31	High	31.42	High
LL	40.65	12.79	Medium	14.89	Medium	73.72	High	22.65	High
LW	1.72	18.06	Medium	19.37	Medium	86.94	High	34.74	High
NTPP	7.26	34.63	High	34.63	High	99.96	High	71.42	High
ST	10.56	18.75	Medium	18.76	Medium	99.98	High	38.69	High
INL	12.29	13.48	Medium	13.58	Medium	98.49	High	27.59	High
NLPP	8.24	16.23	Medium	16.27	Medium	99.44	High	33.39	High
SPL	29.04	12.89	Medium	12.96	Medium	98.82	High	26.43	High
NSPP	146.94	49.40	High	49.49	High	99.65	High	101.74	High
GLW_PP	0.27	74.25	High	74.26	High	99.98	High	153.17	High
GSW_PP	0.65	67.62	High	67.62	High	99.99	High	139.49	High
LDW_PP	0.07	70.18	High	70.35	High	99.51	High	144.42	High
SDW_PP	0.23	76.04	High	76.04	High	100.00	High	156.88	High
LSR	0.31	32.49	High	32.71	High	98.64	High	66.57	High
DMY_PP	0.29	73.57	High	73.57	High	100.00	High	151.77	High
GFY_PP	7.04	28.83	High	28.84	High	99.94	High	59.47	High
CHL	33.43	16.78	Medium	16.88	Medium	98.84	High	34.42	High
Oxalate	1.46	38.15	High	38.57	High	97.83	High	77.85	High
SL	2.49	10.73	Medium	11.83	Medium	82.32	High	20.09	High
SB	1.27	17.16	Medium	17.40	Medium	97.33	High	34.93	High
TSW	1.91	38.77	High	38.84	High	99.65	High	79.84	High

Genetic advance is described as a movement in allele frequency in the superior direction under selection forces, and it is frequently expressed as a percentage of the mean (genetic gain). The level of variation that responds to selection is measured by genetic

advance. Genetic diversity is critical for genetic gain under selection because it illustrates additive variance, which measures additive gene expression.

High heritability observed for the trait Stem Dry Weight per Plant (100%) and Dry matter Yield per Plant (100%) followed by Green Stem Weight per Plant (99.99%), Stem Thickness (99.98%), Green Leaf Weight per Plant (99.98%), Number of Tillers per Plant (99.96%), Green Fodder Yield per Plant (99.94%), Number of Seed per Plant (99.65%),

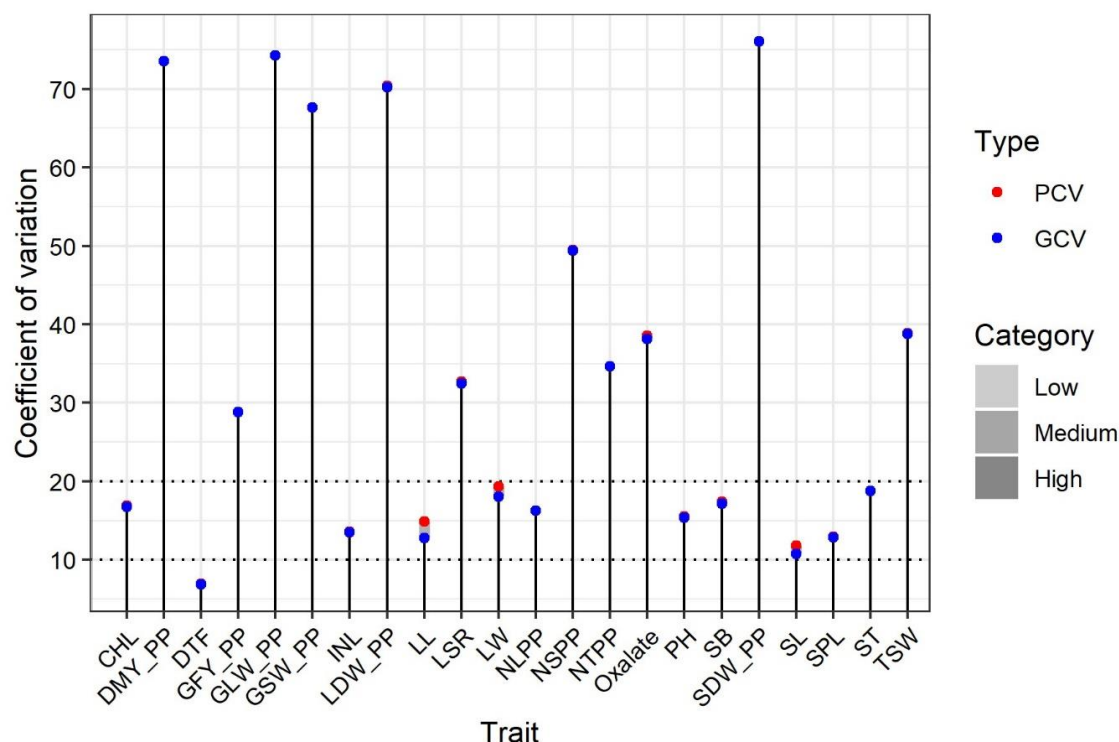


Fig 4.10 (a) Parameters of genetic variability for biomass related traits in Bajra Napier Hybrid

Thousand Seed Weight (99.65%), Leaf Dry Weight pre Plant (99.51%), Number of Leaves per Plant (99.44%), Chlorophyll Content (98.84%), Spike Length (98.82%), Leaf to Stem Ratio (98.64%), Inter Nodal Length (98.49%), Days to 50% Flowering (98.40%), Plant Height (98.31%), Oxalic Acid per cent (97.83%), Seed Breadth (97.33%), Leaf Width (86.94%), Seed Length (82.32%) and Leaf Length (73.72%) (as shown in Fig 4.10 (b)).

High genetic advance over mean had noticed for the trait Stem Dry Weight per Plant (156.88%), Green Leaf Weight per Plant (153.17%), Dry Matter Yield per Plant (151.77%), Leaf Dry Weight per Plant (144.42%), Green Stem Weight per Plant

(139.49%), Number of Seed per Plant (101.74%), Thousand Seed Weight (79.84%), Oxalic Acid per cent (77.85%), Number of Tillers per Plant (71.42%), Leaf to Stem Ratio (66.57%), Green Fodder Yield per Plant (59.47%), Stem Thickness (38.69%), Seed breadth (34.93%), Leaf Width (34.74%), Chlorophyll content (34.42%), Number of Leaves per Plant (33.39%), Plant Height (31.42%), Inter Nodal length (27.59%), Spike Length (26.43%), Leaf Length (22.65%), Seed length (20.09%) and Days to 50% Flowering (14.01%).

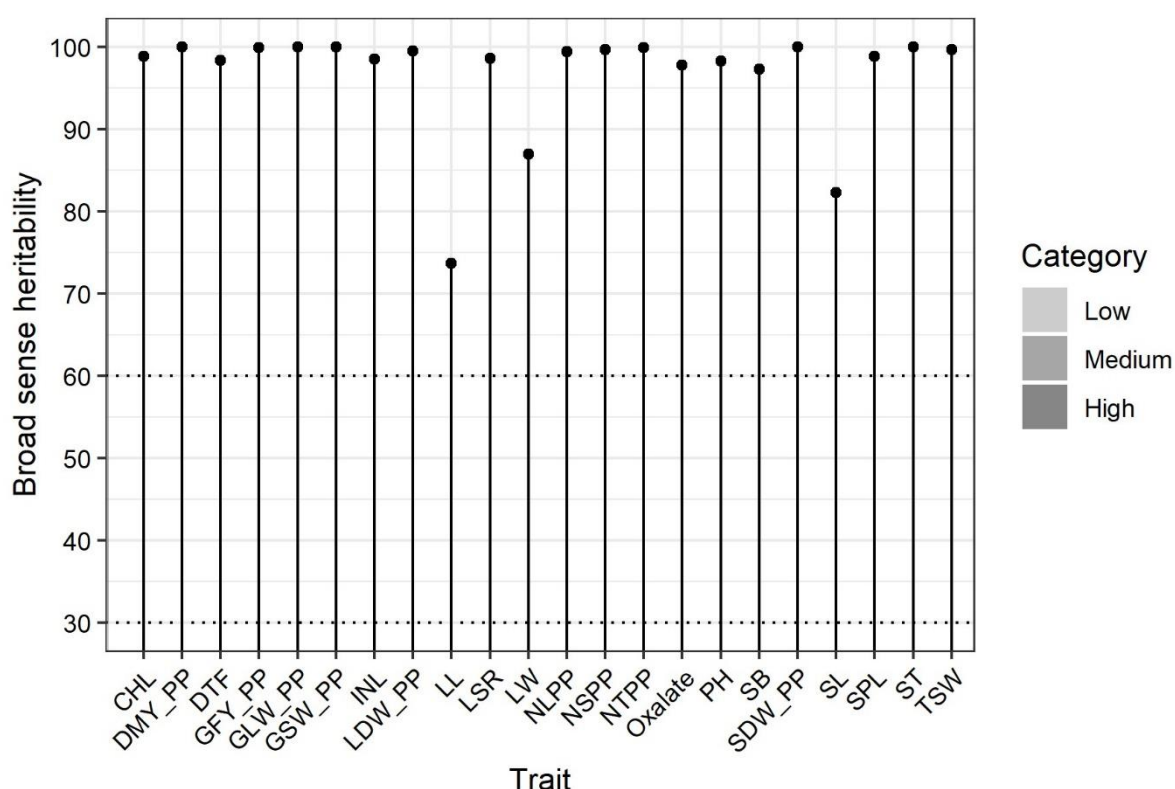


Fig 4.10 (b) Broad sense Heritability for biomass related traits in Bajra Napier Hybrid

All the recorded traits showed high heritability (broad sense) coupled with high genetic advance indicated that these traits can be improved by effective selection measure. Broad sense high heritability along with low genetic advance over mean indicates additive gene action might be expressing by these characters, could be improved by adoption of effective plant breeding programmes. The traits exhibiting moderate heritability (broad sense) and low genetic advance indicates the presence of non-additive gene action could be restricted by implementing effective plant breeding strategies. Similar results were

observed by Kumari *et al.*, (2018); Singh *et al.*, (2020) and Turano *et al.*, (2016) in Napier and Bajra Napier Hybrid.

4.2.1.5 Correlation coefficient analysis

In plant breeding, changes in one variable are generally effects the changes in another. The correlation is due to the combined or net influence of segregating genes, which modified both the gene and its expression. It is always necessary to have information on the nature of the relation between traits and economic yield to facilitate the selection method. The correlation coefficient (r) varied from -1 to +1. Correlation coefficients are used to measure degree and direction of relationship between two and more variables. By virtue of the complexity of yield trait, it becomes essential to measure association between yield and yield attributing (agro-morphological) traits. Positive correlation is a relationship between two variables in which both the variables are increase and decrease in same direction. In negative correlation, both the variables move in opposite direction if one increasing the other is decreasing. Under the present study, the correlation coefficients for 22 biomass related traits were estimated at both genotypic and phenotypic level which is presented in Fig.4.11.

Green Fodder Yield per Plant:

The trait Green Fodder per Plant showed significant positive association with the traits Number of Tillers per Plant (0.23***), Stem Thickness (0.26***), Dry Matter Yield per Plant (0.25***), Stem Dry Weight per Plant (0.25***), Green Stem Weight per Plant (0.24***), Leaf Dry Weight per Plant (0.22***), Green Leaf Weight per Plant (0.21***), Number of Leaves per Plant (0.17**), Leaf Width (0.13*) and Leaf Length (0.13*). Whereas, it exhibits significant negative correlation with Leaf to Stem Ratio (-0.15*).

Spike Length:

The trait Spike Length showed significant positive correlation with the traits Plant Height (0.61***), Leaf Width (0.57***), Leaf Length (0.50***), Stem Thickness (0.50***), Green Stem Weight per Plant (0.46***), Green Leaf Weight per Plant (0.46***), Dry Matter Yield per Plant (0.41***), Stem Dry Weight per Plant (0.40***), Leaf Dry Weight per Plant (0.40***), Number of Leaves per Plant (0.22***), Number of Tillers per Plant (0.21***) and Thousand Seed Weight (0.16***) whereas, it exhibits significant negative correlation with Days to 50% Flowering (-0.46***).

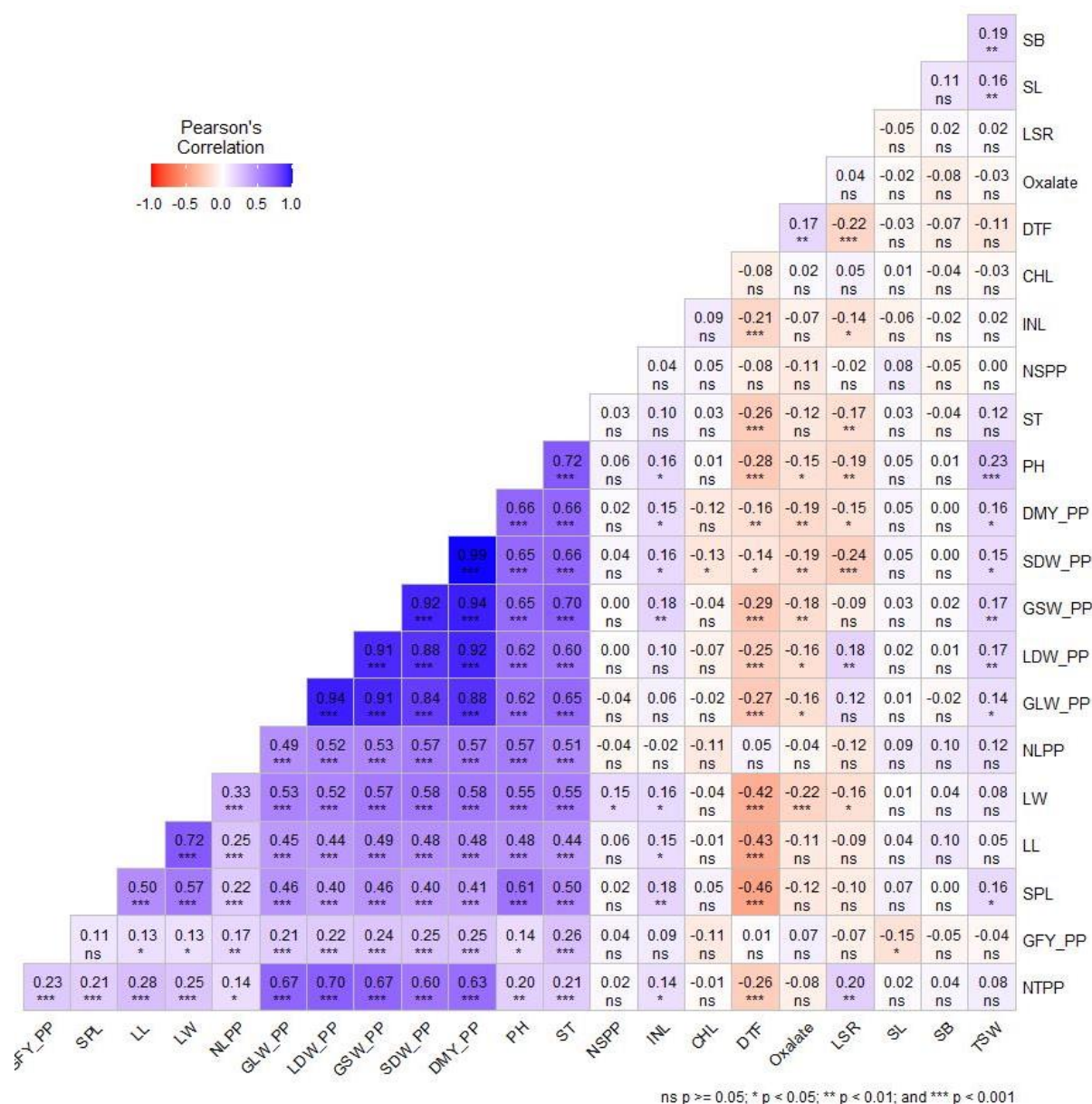


Fig 4.11 Correlation coefficients for 22 biomass related traits in Bajra Napier Hybrid

Leaf Length:

The trait Leaf Length showed significant positive correlation with the traits Leaf Width (0.72***), Spike Length (0.50***), Green Stem Weight per Plant (0.49***), Stem Dry Weight per Plant (0.48***), Dry Matter Yield per Plant (0.48***), Plant Height (0.48***), Green Leaf Weight per Plant (0.45***), Leaf Dry Weight per Plant (0.44***), Stem Thickness (0.44***), Number of Tillers per Plant (0.28***), Number of Leaves per Plant (0.25***), Inter Nodal Length (0.15*) and Green Fodder Yield per Plant (0.13*)

whereas, it showed significant negative correlation with the traits Days to 50% Flowering (-0.43***).

Leaf Width:

The trait Leaf Width showed significant positive correlation with the traits Leaf Length (0.72), Stem Dry Weight per Plant (0.58***), Dry Matter Yield per Plant (0.58***), Green Stem Weight per Plant (0.57***), Spike Length (0.57***), Plant Height (0.55***), Stem Thickness (0.55***), Green Leaf Weight per Plant (0.53***), Leaf Dry weight per Plant (0.52***), Number of Leaves per Plant (0.33***), Number of Tillers per Plant (0.25***), Inter Nodal Length (0.16*), Number of Seed per Plant (0.15*) and Green Fodder Yield per Plant (0.13*) whereas, it showed significant negative correlation with the traits Days to 50% Flowering (-0.42***), Oxalic acid per cent (-0.22***) and Leaf to Stem Ratio (-0.16*).

Number of Leaves per Plant:

The trait Number of Leaves per Plant showed significant positive correlation with the traits Stem Dry Weight per Plant (0.57***), Dry Matter Yield per Plant (0.57***), Plant Height (0.57***), Green Stem Weight per Plant (0.53***), Leaf Dry Weight per Plant (0.52***), Stem Thickness (0.51***), Green Leaf Weight per Plant (0.49***), Leaf Width (0.33***), Leaf Length (0.25***), Spike Length (0.22***), Green Fodder Yield per Plant (0.17***) and Number of Tillers per Plant (0.14***).

Green Leaf Weight per Plant:

The trait Green Leaf Weight per Plant showed significant positive correlation with the traits Leaf Dry Weight per Plant (0.94***), Green Stem Weight per Plant (0.91***), Dry Matter Yield per Plant (0.88***), Stem Dry Weight per Plant (0.84***), Number of Tillers per Plant (0.67***), Stem Thickness (0.65***), Plant Height (0.62***), Leaf Width (0.53***), Number of Leaves per Plant (0.49***), Spike Length (0.46***), Leaf Length (0.45***) and Green Fodder Yield per Plant (0.21***) whereas, it showed significant negative correlation with the traits Days to 50% Flowering (-0.27***) and Oxalic acid per cent (0.16*).

Leaf Dry Weight per Plant:

The trait Leaf Dry Weight per Plant showed significant positive correlation with the traits Green Leaf Weight per Plant (0.94**), Dry Matter Yield per Plant (0.92***),

Green Stem Weight per Plant (0.91***), Stem Dry Weight per Plant (0.88***), Number of Tillers per Plant (0.70***), Plant Height (0.62***), Stem Thickness (0.60***), Number of Leaves per Plant (0.52***), Leaf Width (0.52***), Leaf Length (0.44***), Spike Length (0.40***), Green Fodder Yield per Plant (0.22***), Leaf to Stem Ratio (0.18***), and Thousand Seed Weight (0.17***), whereas, it showed significantly negative correlation with the traits Days to 50% Flowering (-0.25***), and Oxalic acid per cent (-0.16*).

Green Stem Weight per Plant:

The trait Green Stem Weight per Plant showed significant positive correlation with the traits Dry Matter Yield per Plant (0.94***), Stem Dry Weight per Plant (0.92***), Leaf Dry Weight per Plant (0.91***), Green Leaf Weight per Plant (0.91***), Stem Thickness (0.70***), Number of Tillers per Plant (0.67***), Plant Height (0.65***), Leaf Width (0.57***), Number of Leaves per Plant (0.53***), Leaf Length (0.49***), Spike Length (0.46***), Green Fodder Yield per Plant (0.24***), Inter Nodal Length (0.18**) and Thousand Seed Weight (0.17**). Whereas it showed negative significant correlation with the traits Days to 50% Flowering (0.29***), and Oxalic acid per cent (0.16**).

Stem Dry Weight per Plant:

The trait Stem Dry Weight per Plant showed significant positive correlation with the traits Dry matter Yield per Plant (0.99***), Green Stem Weight per Plant (0.92***), Leaf Dry Weight per Plant (0.88***), Green Leaf weight per Plant (0.84***), Stem Thickness (0.66***), Plant Height (0.65***), Number of Tillers per Plant (0.60***), Leaf Width (0.55***), Number of Leaves per Plant (0.57***), Leaf Length (0.48***), Spike Length (0.40***), Inter Nodal Length (0.16*) and Thousand Seed Weight (0.15*) whereas, it showed significant negative correlation with the traits Leaf to Stem Ratio (-0.24***), Oxalic acid content (-0.19**), Days to 50% Flowering (-0.14*) and Chlorophyll content (-0.13*).

Dry matter Yield per Plant:

The trait Dry Matter Yield per Plant showed significant positive correlation with the traits Stem Dry Weight per Plant (0.99***), Green Stem Weight per Plant (0.94***), Leaf Dry Weight per Plant (0.92***), Green Leaf Weight per Plant (0.88***), Plant Height (0.66***), Stem Thickness (0.66***), Number of tillers per Plant (0.63***), Leaf Width (0.58***), Number of Leaves per Plant 90.57***), Leaf Length (0.46***), Spike Length

(0.41***), Green Fodder Yield per Plant (0.25***), Thousand Seed Weight (0.16*) and Inter Nodal Length (0.15*) whereas significant negative correlation was found with the traits Oxalic acid content (-0.19**), Days to 50% Flowering (-0.16**) and Leaf to Stem ratio (-0.15*).

Plant Height:

The trait Plant Height showed significant positive correlation with the traits Stem Thickness (0.72***), dry matter Yield per Plant (0.66***), Stem Dry weight per Plant (0.654***), Green Stem Weight per Plant (0.65***), Leaf Dry Weight per Plant (0.62***), Green Leaf Weight per Plant (0.62***), Spike Length (0.61***), Number of Leaves per Plant (0.57***), Leaf Width (0.55***), Leaf length (0.48***), Thousand Seed Weight (0.23***), Number of Tillers per Plant (0.20**), Inter Nodal Length (0.16*) and Green Fodder Yield per Plant (0.14***), whereas, it showed significant negative correlation with the traits Days to 50% Flowering (-0.28***), Leaf to Stem Ratio (0.19**) and Oxalic acid content (-0.15*).

Stem Thickness:

The trait Stem Thickness showed significant positive correlation with the traits Plant Height (0.72***), Green Stem Weight per Plant (0.70***), Dry Matter Yield per Plant (0.66***), Stem Dry Weight per Plant (0.66***), Green Leaf Weight per Plant (0.65***), Leaf Dry weight per Plant (0.60***), Leaf Width (0.55***), Number of Leaves per Plant (0.51***), Spike Length (0.50***), Leaf Length (0.44***), Green Fodder yield per Plant (0.26***), and Number of Tillers per Plant (0.21***), whereas, it showed significant negative correlation with the traits Days to 50% Flowering (-0.26***), and Leaf to Stem ratio (0.17**).

Number of Seeds per Plant:

The trait Number of Seeds per Plant showed significant positive correlation with the trait Leaf Width (0.15*) only.

Inter Nodal Length:

The trait Inter Nodal Length showed significant positive correlation with the traits Green Stem Weight per Plant (0.18**), Spike Length (0.18**), Plant Height (0.16*), Stem Dry Weight per Plant (0.16*), Leaf Width (0.16*), Leaf Length (0.15*), Dry matter Yield per Plant (0.15*) and Number of tillers per Plant (0.14*) whereas, it showed significant

negative correlation with the traits Days to 50 % Flowering (-0.21***) and Leaf to Stem Ratio (-0.14*).

Chlorophyll Content:

The trait Chlorophyll Content showed significant negative correlation with the trait Stem Dry Weight per Plant (-0.13***) only.

Days to 50% flowering:

The trait Days to 50% Flowering showed significant negative correlation with the traits Spike Length (-0.46***), Leaf Length (-0.13***), Leaf width (-0.42***), Green Stem Weight per Plant (-0.29***), Plant Height (-0.28***), Green Leaf Weight per Plant (-0.27***), Number of Tillers per Plant (-0.26***), Leaf Dry weight (-0.25***), Leaf to Stem Ratio (-0.22***), Inter Nodal length (-0.21***), Oxalic acid per cent (-0.17**), Dry Matter Yield per Plant (-0.16**) and Stem Dry Weight (-0.14*).

Oxalic acid per cent:

The trait Oxalic acid per cent showed significant negative correlation with the traits Leaf Width (-0.22***), Dry Matter Yield per Plant (0.19**), Green Stem Weight per Plant (-0.18**), Days to 50% Flowering (-0.17**), Leaf dry Weight (-0.16*), Green Leaf Weight per Plant (-0.16*) and Plant height (-0.15*).

Leaf to Stem Ratio:

The trait Leaf to Stem Ratio showed significant positive correlation with the traits number of tillers per Plant (0.20**) and Leaf dry weight (0.18**) whereas, it showed significant negative correlation with the traits Stem Dry Weight (-0.24***), Days to 50% Flowering (-0.22***), Plant Height (-0.19**), Stem Thickness (-0.17**), Leaf Width (-0.16*), Dry Matter Yield per Plant (-0.15*) and Inter Nodal Length (-0.14*).

Seed Length:

The trait Seed Length showed significant positive correlation with the trait Thousand Seed Weight (0.16**) whereas, it showed significant negative correlation with the trait Green Fodder yield per Plant (-0.15*).

Seed Breadth:

The trait Seed Breadth showed significant positive correlation with the trait Thousand seed Weight (0.19**) only.

Thousand seed Weight:

The trait Thousand Seed Weight showed significant positive correlation with the traits Plant Height (0.23***), Seed Breadth (0.19**), Green Stem Weight per Plant (0.17**), Leaf Dry Weight per Plant (0.17**), Dry Matter Yield per Plant (0.16*), Seed Length (0.16**), Stem Dry Weight per Plant (0.15*) and Green Leaf Weight per Plant (0.14*).

Likewise positive correlation of GFY with plant height and leaf breadth was found by Turano *et al.*, (2016). Similarly, GFY was found positively correlated with leaf area, number of leaves and plant height, whereas negatively correlated with number of tillers per plant by Singh *et al.*, 2020.

4.2.1.6 Principal Component Analysis and Biplot analysis:

Out of the 22 traits studied, only six principal components (PCs) exhibited more than 1.00 eigen value and showed about 71.97% of the total cumulative variability among the traits studied (as shown in Fig.4.12(a)). The scree plot exhibited the percent of variation by each PCs and deduced that PC1 has a preponderance of maximum variability of about 35.47% with the highest eigen value. Further, the progressive decrease in variability was found in PC2, PC3, PC4, PC5 and PC6 with values 8.23, 7.18, 6.43, 5.05, 4.89 and 4.68% respectively. Fig.4.12 (a) indicated that the maximum variation was observed in PC1 compared to the other 22 PCs. So, the selection of lines and traits from this PC will be useful. The PCA of 250 immortalised F₂ populations extracted two major principal components (eigenvalues > 1) that accounted collectively for 35.47 and 8.23 per cent of the variance. Principal component 1 (PC1, X-axis, Fig.4.12 (b)) explained 35.47 per cent of the data set variation, while PC2 (Y-axis) explained 8.23 per cent of the data set variation, for all the measured traits.

PC1 includes the traits with highest positive value for Days to 50% Flowering, Leaf to Stem Ratio, Chlorophyll content and Oxalic acid per cent; PC2 contains the traits Day to 50% Flowering, Number of Tillers per Plant, Number of Leaves per Plant, Green Leaf Weight per Plant, Green Stem Weight per Plant, Leaf Dry Weight per plant, Stem Dry

Weight per Plant, Leaf to Stem Ratio, Dry matter Yield per Plant, Green Fodder yield per Plant and Oxalic acid per cent; while, PC3 includes the traits Number of tillers per Plant, Inter Nodal Length, Green Leaf Weight per Plant, Green Stem Weight per Plant, Leaf Dry Weight per Plant, Leaf to Stem Ratio, Chlorophyll content, Seed Breadth and Thousand Seed Weight; PC4 includes the traits Leaf Length, Leaf Width, Number of Tillers per Plant, Stem Thickness, Inter Nodal length, Spike Length, Number of Seeds per Plant, Green Stem Weight per Plant, Seed Dry Weight per Plant, Green Fodder Yield per Plant, Chlorophyll content and Oxalic acid per cent;

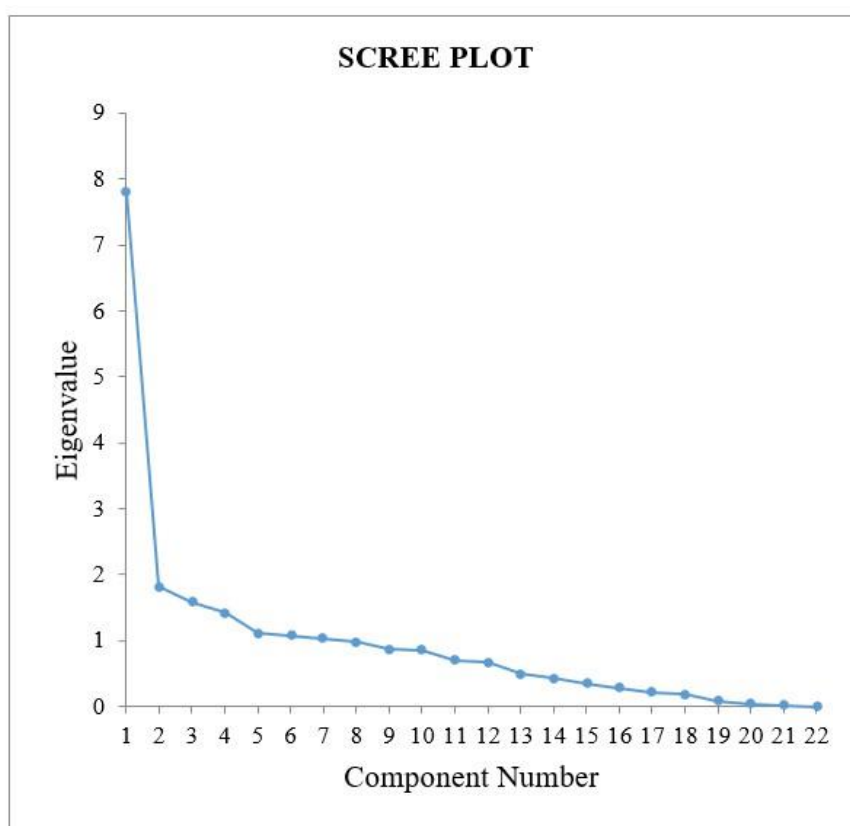


Fig 4.12 (a) Scree plot of principal component analysis of Bajra Napier Hybrid immortalised F₂ population

PC5 contains the traits days to 50% Flowering, Leaf Width, Number of Tillers per Plant, Inter Nodal Length, Number of Seed per Plant, Green Stem Weight per Plant, Stem Dry Weight per Plant, Dry matter Yield per Plant, Seed Length and Seed Breadth; PC6 includes the traits Leaf Length, leaf Width, Number of tillers per Plants, Inter Nodal length, Spike Length, Green Fodder Yield per Plant, Seed Breadth and Thousand Seed Weight; PC7 includes the traits Plant Height, Leaf Length, Leaf Width, Stem Thickness,

Number of Leaves per Plant, Spike Length, Number of Seed per Plant, Green leaf Weight per plant, Leaf Dry Weight per Plant and Leaf to Stem Ratio (as shown in Table 4.7).

PC1 and PC2 contributes for majority of the variation found in the population. Selection based on the parameters (traits) falls under these two components would be awarding to screen the population. These principal components simplifies the screening procedures instead of screening for all the traits one can go for these principal components in order to evaluate the biomass related traits in Bajra Napier hybrid. Biplot analysis from the Fig. 4.12 (c) showed the amount of variation present among the population in relation to these principal components.

Table 4.7 Interpretation of rotated component matrix for the traits having highest value (>1.0) in each PCs

PC1	PC2	PC3	PC4	PC5	PC6	PC7
DTF	DTF	NTPP	LL	DTF	LL	PH
LSR	NTPP	INL	LW	LW	LW	LL
CHL	NLPP	GLW_PP	NTPP	NTPP	NTPP	LW
Oxalate	GLW_PP	GSW_PP	ST	INL	INL	ST
	GSW_PP	LDW_PP	INL	NSPP	SPL	NLPP
	LDW_PP	LSR	SPL	GSW_PP	GFY_PP	SPL
	SDW_PP	CHL	NSPP	LDW_PP	SB	NSPP
	LSR	SB	GSW_PP	SDW_PP	TSW	GLW_PP
	DMY_PP	TSW	SDW_PP	DMY_PP		LDW_PP
	GFY_PP		GFY_PP	SL		LSR
	Oxalate		CHL	SB		
			Oxalate			

4.2.1.7 Cluster Analysis:

Cluster analysis clustered 250 immortalized F₂ lines into three clusters. According to dendrogram Cluster I includes both the parents *i.e.*, Tetra Bajra and FD-475 (Napier) along with the genotypes. Dendrogram showed the extant of variation present in immortalised F₂ population. Such type of variation does not existed previously in this crop due to the presence of sterility. Therefore, such population will be used for variability creation in this BN hybrid crop.

4.3 QTLs for biomass related traits in Bajra Napier Hybrid

To understand the genetic and molecular basis of biomass related traits, developed genetic maps and extensive phenotyping data generated on immortalized F₂ population were analysed for identification of QTLs.

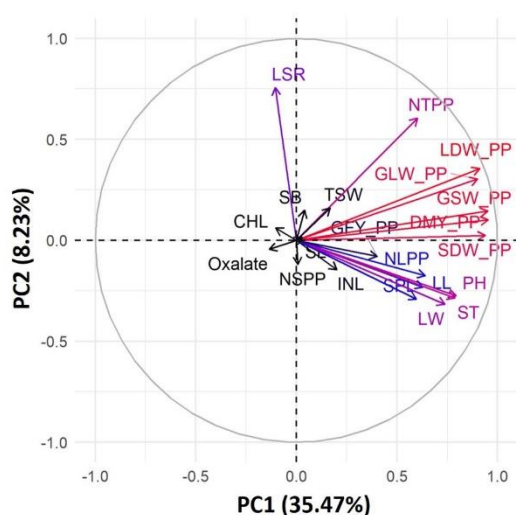


Fig. 4.12(b)

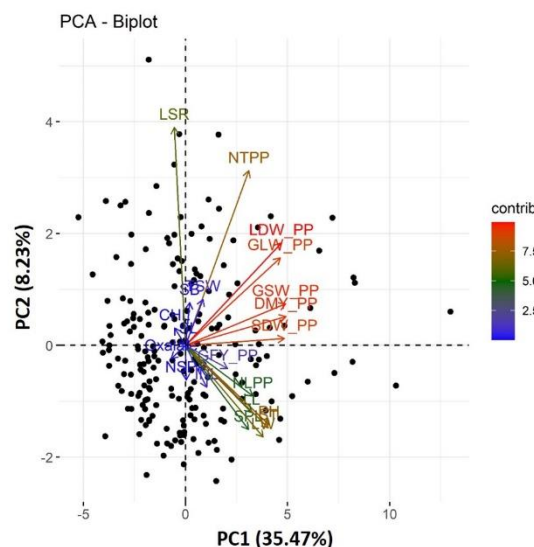


Fig. 4.12(c)

Fig. 4.12 (b) Principal component analysis of measured biomass related traits in immortalized F₂ population (c) Bi-plot analysis of measured biomass related traits in immortalized F₂ population

A total of 33 QTLs ($\text{LOD} \geq 3.0$) were detected from 250 F₂ lines for 13 traits measured, with QTL detected on all the linkage groups except LG7 (Table 4.8, Fig. 4.13). In total, 5 QTLs were detected for morphological traits (one for Plant Height, one for Number of Tillers per Plant, two for Inter Nodal length and one for Spike Length), 4 for phenological traits (Days to 50% Flowering), 5 for anti-nutritional factors (all five for Oxalic acid per cent), 19 for yield related traits (two for Green Fodder Yield per Plant, two for Green leaf Weight per Plant, two for Green Stem Weight per Plant, two for Leaf Dry Weight per Plant, two for Stem Dry weight per Plant, eight for number of Seed per Plant and one for Seed Breadth).

LG1 contained a total of 14 QTLs (three for Days to 50% Flowering, two for Oxalic acid per cent, one each for Inter Nodal length, Green Fodder Yield per Plant, Green Leaf Weight per Plant, Green Stem weight per Plant, Leaf Dry Weight per Plant, Stem Dry Weight per Plant, Spike Length, Number of Seed per Plant and Seed breadth); LG2 contained a total of 11 QTLs (three for Number of Seeds per Plant, one each for Days to 50% Flowering, Plant Height, Inter Nodal length, Green Fodder Yield per Plant, Green Leaf Weight per Plant, Green Stem Weight per Plant, Leaf Dry weight per Plant and Stem Dry Weight per Plant) while LG3 and LG4 contained only one QTL for Oxalic acid per cent; LG5 contained only three QTLs for Number of Seed per Plant; LG6 contained only

two QTLs (one each for Oxalic acid per cent and Number of Seed per Plant); LG7 has no QTLs detected on it.

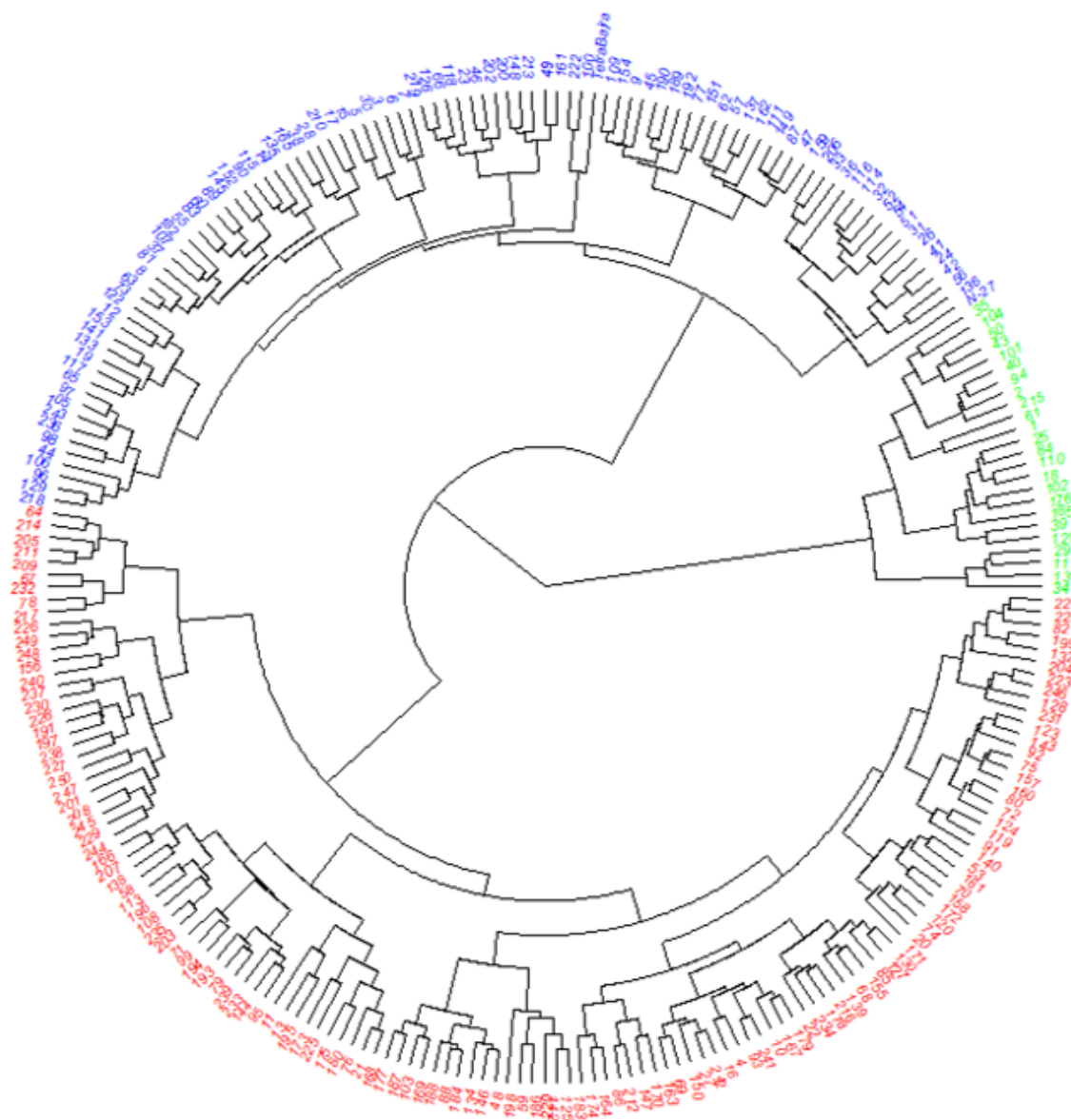


Fig 4.13 Spherical dendrogram for 250 immortalized F₂ lines of Bajra Napier Hybrid showing extent of variation present among the population

4.3.1 Trait dissection

Comprehensive QTL analysis provided an opportunity to analyse biomass related traits in depth. As QTL analysis was undertaken based on phenotypic data for 22 traits and collected during whole year at particular geographical location. Phenotypic variation explained (PVE) by QTLs ranged from 10.8 to 70.6 per cent (as shown in Table 4.8). The

QTLs with PVE less than 10% were not considered. The highest phenotypic variation (70.6%) was explained by the QTL for Spike Length. Although QTLs were detected on all linkage groups except LG7, six regions on LG1, LG2 and LG5 contained QTLs for several traits and may have contained QTLs with pleiotropic effects (as shown in Fig. 4.14).

4.3.1.1 Morphological traits

Of the eight morphological traits analysed, significant QTLs were identified for four traits *viz.* two for Inter Nodal length and one each for Plant Height, Number of tillers per Plant and Spike Length. Thus, five QTLs were detected for morphological traits using composite interval mapping (as shown in Table 4.8 and Fig. 4.14). Further of the 2 QTLs for INL, one QTL was present on LG2 and one on LG1 with PVE ranging from 10.8-11.1 per cent at LOD value of 3.9-4.7. Subsequently, one QTL for Plant height was on LG2 with PVE 32.3 per cent at LOD value of 3.33, one QTL for Number of Tillers per Plant was on LG5 with PVE 41.7 per cent at LOD value of 8.50 and one QTL for Spike length was on LG1 with PVE 70.6 per cent at LOD value of 3.49.

4.3.1.2 Phenological traits:

The one phenological trait measured under this study was Days to 50% flowering. A total of four QTLs were identified for this trait whereas three of these were detected on LG1 with PVE ranging from 16.12-17.53 per cent at LOD value of 3.32-3.70 and one QTL was detected on LG2 with PVE 22.63 per cent at LOD value of 3.67.

4.3.1.3 Physiological traits:

Of the two physiological traits measured, significant QTLs were identified for only one trait *i.e.*, Oxalic acid per cent. A total of six QTLs were identified for this trait on five different linkage groups. Two of these were detected on LG1 with PVE ranging from 15.54-16.20 per cent at LOD value of 3.43-3.74, one was identified on LG3 with PVE of 12.64 at LOD value of 4.54, one was on LG4 with PVE 19.51 per cent at LOD value of 3.77 and another was on LG6 with PVE 23.78 per cent at LOD value of 3.49.

4.3.1.4 Yield and related traits:

Of the eleven yield related traits analysed, significant QTLs were identified for only seven trait *viz.* eight for the trait Number of Seeds per Plant, two for each Green Fodder Yield per Plant, Green Leaf Weight per Plant, Green Stem Weight per Plant, Leaf

Dry Weight per Plant, Stem Dry Weight per Plant and one for Seed Breadth. Thus a total of 19 QTLs were mapped for yield and related traits. Further of the eight QTLs for NSPP, three were detected on LG2 with the PVE ranging from 11.71-12.00 per cent at LOD value of 4.50-6.73, three were on LG5 with the PVE ranging from 11.57-12.83 per cent at LOD value of 7.28-7.79, one was on LG1 with the PVE 10.83 per cent at LOD value of 5.24 and one was on LG6 with the PVE 11.57 per cent at LOD value of 5.71. For Green Fodder Yield per Plant, Green Leaf Weight per Plant, Green Stem Weight per Plant, Leaf Dry Weight per Plant and Stem Dry Weight per Plant, one QTL was detected on LG1 with the PVE 11.06 per cent at LOD value of 3.99 and another QTL was identified on LG2 with the PVE 10.8 per cent at LOD value of 4.76. For the trait Seed Breadth, only one QTL was detected on LG1 with the PVE 58.09 per cent at LOD value of 5.61.

4.4 Future research prospects

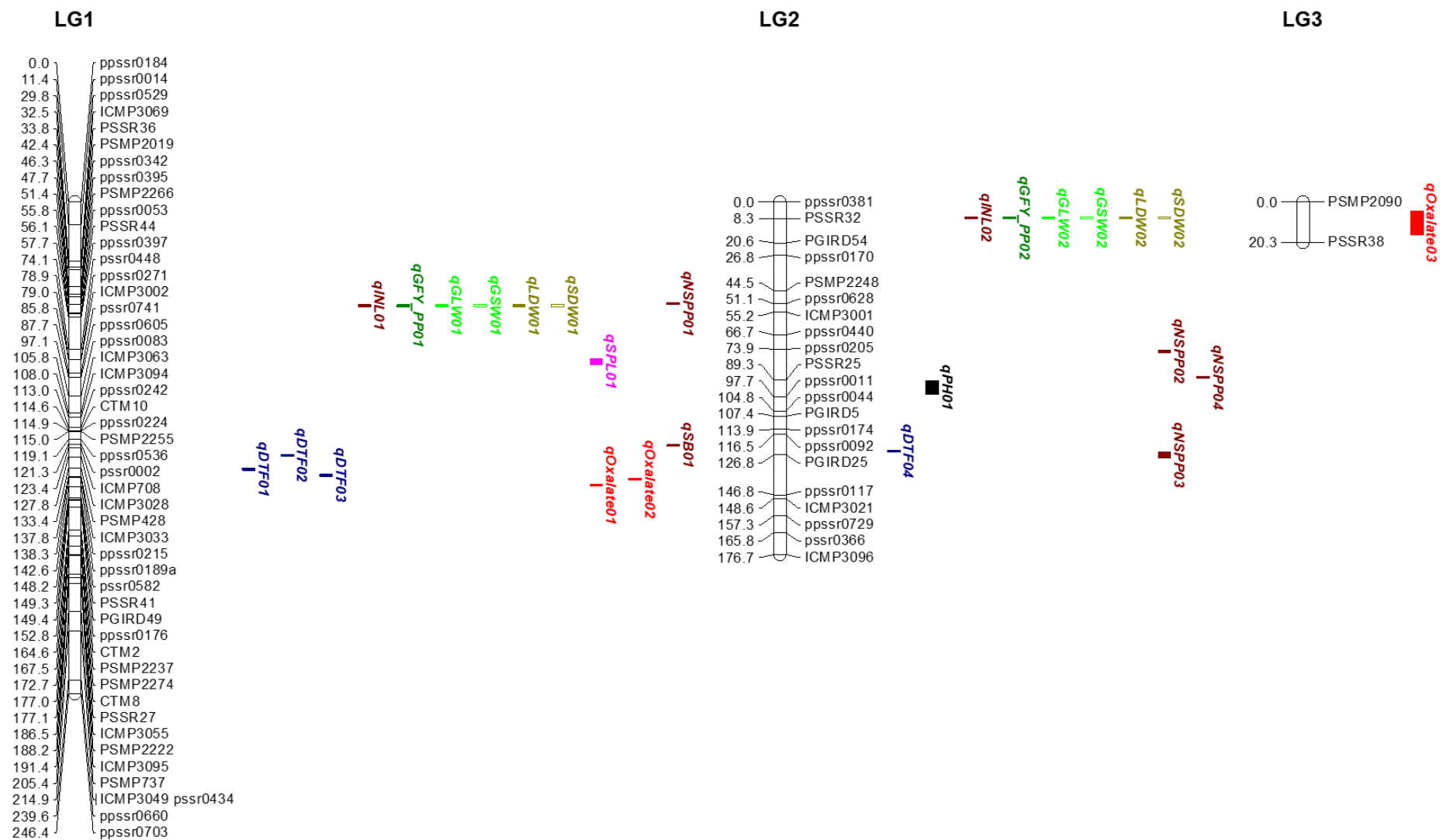
Future research work arising from this thesis should be directed towards the following areas:

- a. The first-ever framework linkage map of Bajra Napier Hybrid constructed in this study opens a window for the exploration of molecular breeding work in this crop.
- b. The markers distributed over the linkage map may act as anchor markers for further development of consensus map in this crop using advance mapping populations like RILs, NILs, BILs etc.
- c. The first-ever QTL map developed from this study will also help in understanding marker-trait association studies which further opens era of MAS in this crop
- d. The present study revealed the mapping of 33 QTLs for 13 biomass related traits, which provides the road map for the further discoveries in the crop
- e. The transgressive segregants identified in this study will be helpful for generating variation in this crop.

Table 4.8 QTLs for biomass related traits identified using QTL Cartographer

Immortalized F₂ lines (<i>C. americanus</i> X <i>C. purpureus</i>)								
Trait Name	Chromosome	Position	Left Marker	Right Marker	LOD	PVE (%)	Add	Dom
Morphological traits								
PH	LG2	91	PSSR25	ppssr0011	3.333	32.329	2.3226	35.1754
NTPP	LG5	66	ICMP3081	ppssr0162	8.504	41.662	0.0725	4.6779
INL	LG2	8	ppssr0381	PSSR32	4.761	10.8	0.019	5.9867
INL	LG1	52	PSMP2266	ppssr0053	3.9935	11.067	-0.0495	2.9095
SPL	LG1	79	ICMP3002	psr0741	3.4965	70.64	1.3387	0.9736
Phenological traits								
DTF	LG1	134	PSMP428	ICMP3033	3.3292	16.12	0.2269	10.2249
DTF	LG1	127	ICMP708	ICMP3028	3.7038	17.11	-1.52	9.1948
DTF	LG1	137	PSMP428	ICMP3033	3.358	17.535	0.0215	10.1821
DTF	LG2	125	ppssr0092	PGIRD25	3.6723	22.636	1.1158	9.6597
Anti nutritional factors								
Oxalate	LG3	10	PSMP2090	PSSR38	4.5434	12.649	0.0313	0.7301
Oxalate	LG1	142	ppssr0215	ppssr0189a	3.4347	15.54	-0.0007	0.825
Oxalate	LG1	139	ppssr0215	ppssr0189a	3.7476	16.207	-0.0017	0.8373
Oxalate	LG4	62	ICMP3020	CTM12	3.7737	19.512	-0.0748	1.0252
Oxalate	LG6	62	ICMP3068	ppssr0328	3.497	23.74	0.0472	0.9116
Yield and yield related traits								
GFY_PP	LG2	8	ppssr0381	PSSR32	4.761	10.8	0.019	5.9867
GFY_PP	LG1	52	PSMP2266	ppssr0053	3.9935	11.067	-0.0495	2.9095
GLW	LG2	8	ppssr0381	PSSR32	4.761	10.8	0.019	5.9867
GLW	LG1	52	PSMP2266	ppssr0053	3.9935	11.067	-0.0495	2.9095
GSW	LG2	8	ppssr0381	PSSR32	4.761	10.8	0.019	5.9867

GSW	LG1	52	PSMP2266	ppssr0053	3.9935	11.067	-0.0495	2.9095
LDW	LG2	8	ppssr0381	PSSR32	4.761	10.8	0.019	5.9867
LDW	LG1	52	PSMP2266	ppssr0053	3.9935	11.067	-0.0495	2.9095
SDW	LG2	8	ppssr0381	PSSR32	4.761	10.8	0.019	5.9867
SDW	LG1	52	PSMP2266	ppssr0053	3.9935	11.067	-0.0495	2.9095
NSPP	LG1	51	ppssr0395	PSMP2266	5.2418	10.833	5.4796	147.2642
NSPP	LG6	50	ppssr0364	ICMP3068	5.7108	11.571	3.1075	165.7724
NSPP	LG5	61	ICMP3081	ppssr0162	7.28	11.577	-1.0355	167.7024
NSPP	LG5	83	ICMP3081	ppssr0162	7.2822	11.584	-1.1913	167.5307
NSPP	LG2	75	ppssr0205	PSSR25	5.6627	11.712	-0.2408	157.8442
NSPP	LG2	126	ppssr0092	PGIRD25	4.5005	12.003	6.3719	149.8239
NSPP	LG2	88	ppssr0205	PSSR25	6.7336	12.003	3.6483	158.6188
NSPP	LG5	54	ppssr0058	ICMP3081	7.7927	12.837	3.7031	172.9583
SB	LG1	122	pssr0002	ICMP708	5.6181	58.095	-0.1046	0.1818



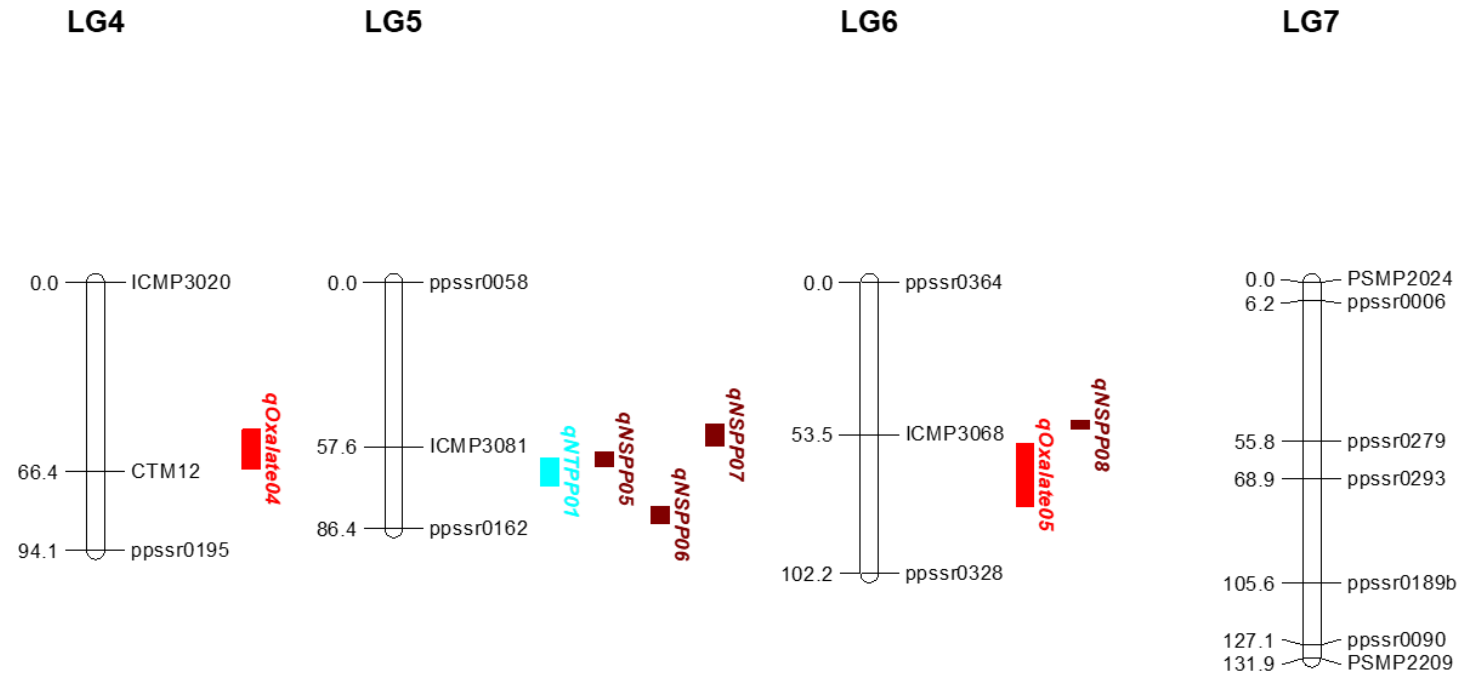


Fig 4.14 Likelihood intervals for quantitative trait loci associated with 13 biomass related traits in immortalized F₂ population of the cross between *C. americanus* x *C. purpureus*

The present investigation entitled, “Construction of framework linkage map and genetic dissection of biomass related traits in Bajra-Napier Hybrid” was undertaken to construct first-ever interspecific framework linkage map and to identify genomic regions linked with biomass related traits in Bajra Napier Hybrid.

The study was carried out using immortalized F₂ population of 250 individuals derived from an interspecific cross of *Cenchrus americanus* x *Cenchrus purpureus*. The immortalized F₂ population was phenotyped for 22 biomass related traits at Central Research Farm, ICAR-IGFRI, Jhansi. Field phenotyping was carried out in augmented randomized complete block design (RCBD).

A first ever framework linkage map of Bajra Napier Hybrid was constructed using an interspecific immortalised F₂ mapping population (*Cenchrus americanus* x *Cenchrus purpureus*) using SSR markers. A total of 217 SSR markers [41 SSRs from Azevedo *et al.* (2012); 56 SSRs from Kendel *et al.* (2016); 87 SSRs from Lopez *et al.* (2018) and 33 SSRs from Zhou *et al.* (2018)] were used to identify polymorphic primers between *Cenchrus americanus* x *Cenchrus purpureus*, the parental lines of the mapping population. Of the 217 SSR primer pairs, 138 (63.5 %) primer pairs were found to be polymorphic and were further validated with 14 randomly selected F₂ lines to confirm the polymorphism among the population. A total of 100 markers were selected after validation and assigned map positions at LOD 4.0 on seven linkage groups, spanning 858.08 cM distance of the Bajra Napier Hybrid genome with an average marker density of 9.75 cM. These markers exhibited a non-random distribution varying in density from 5.02 cM/locus to 34.06 cM/locus on seven LGs. The SSR markers mapped on the present map included a total of 88 markers. Of these, 12 loci (12 %) showed segregation distortion.

Further, the linkage map constructed was used for the identification of QTLs related to biomass related traits. Analysis of variance revealed significant differences for all the 22 measured traits between the *Cenchrus americanus* x *Cenchrus purpureus*. The ANOVA of 250 immortalized F₂ revealed significant differences for all the traits. Significant effect of environment was also observed for few of the traits measured like LL, LW and SL.

The PCA of immortalized F_2 populations extracted two major principal components (eigenvalues > 1) that accounted collectively for 37.72 and 8.14 per cent of the variance for the biomass related traits. Selection for these traits covered under the PC1 and PC2 would be rewarding.

A significant but negative correlation was found for spike length, leaf length, leaf width, green leaf weight per plant, leaf dry weight per plant, green stem weight per plant, plant height, stem thickness and inter nodal length with Days to 50% flowering. Similarly, strong and positive association was found among stem dry weight per plant, dry matter yield per plant, green stem weight per plant, green leaf weight per plant and plant height. This association was further supported by the presence of high and positive correlation between them.

A total of 33 QTLs ($LOD \geq 30$) were detected for biomass related traits. Of all the 22 measured traits, QTLs were detected for 13 traits only. Overall, 5 QTLs were detected for morphological traits, 4 for phenological traits, 5 for oxalate content and 19 for yield related traits. Fourteen QTLs were located on LG1 followed by eleven on LG2, one on each LG3 and LG4, 3 on LG5 and 2 on LG6. Phenotypic variation explained by QTLs ranged from 10.8 to 70.64 per cent. The highest phenotypic variation (70.64 %) was explained by the QTL for spike length.

LG1 contained a total of 14 QTLs (three for Days to 50% Flowering, two for Oxalic acid per cent, one each for Inter Nodal length, Green Fodder Yield per Plant, Green Leaf Weight per Plant, Green Stem Weight per Plant, Leaf Dry Weight per Plant, Stem Dry Weight per Plant, Spike Length, Number of Seed per Plant and Seed breadth); LG2 contained a total of 11 QTLs (three for Number of Seeds per Plant, one each for Days to 50% Flowering, Plant Height, Inter Nodal length, Green Fodder Yield per Plant, Green Leaf Weight per Plant, Green Stem Weight per Plant, Leaf Dry weight per Plant and Stem Dry Weight per Plant) while LG3 and LG4 contained only one QTL for Oxalic acid per cent; LG5 contained only three QTLs for Number of Seed per Plant; LG6 contained only

two QTLS (one each for Oxalic acid per cent and Number of Seed per Plant); LG7 has no QTLs detected on it.

Evidently, the map generated in the present study although covered a significant portion of the genome, a further saturation of this map with additional markers (SSRs or SNPs) is imperative for its efficient utilization. With the long-term goal of understanding the genetic basis of biomass related traits, the present study was focused on identification of major QTLs for 13 traits in Bajra Napier Hybrid. In conclusion, it is envisaged that the present linkage map, fortified with 88 SSR markers and 33 QTLs for biomass related traits would provide a means to breeders for further genetic enhancement of this crop species. However, a denser genetic linkage maps with large number of markers with the inclusion of SNPs would facilitate the identification of more resolved and fine QTL positions which can significantly improve the resolution of identified QTLs for mapping. The identification of QTLs controlling agronomically important traits would improve our genetic understanding of these traits and finally provide the basis for MAS of these traits. Therefore, QTL validation and fine mapping is the next step towards successful application of these findings in MAS.

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Appendix-I

Mean performance of 250 immortalised F₂ lines of Bajra Napier Hybrid along with parents for biomass related traits

F ₂ Lines	DTF	PH	LL	LW	NTPP	ST	INL	NLPP	SPL	NSPP	GLW_PP	GSW_PP
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
F ₂ -1	87.00	200.60	62.66	2.50	17.00	11.28	19.62	9.60	33.20	101.00	0.67	2.09
F ₂ -2	87.00	206.40	54.44	2.62	12.40	15.72	13.08	11.60	34.80	142.00	0.64	1.85
F ₂ -3	85.00	225.60	43.18	2.06	9.80	11.43	15.72	9.80	30.80	104.00	0.38	1.49
F ₂ -4	88.00	141.20	48.78	1.56	8.00	8.64	14.68	7.20	26.40	200.00	0.12	0.34
F ₂ -5	87.00	218.40	50.82	2.16	7.20	11.43	17.92	9.00	34.00	122.00	0.26	0.92
F ₂ -6	93.00	201.40	50.22	2.14	12.60	11.00	15.70	8.60	33.60	105.00	0.51	1.50
F ₂ -7	87.00	211.00	49.34	2.22	11.60	11.22	18.52	8.80	32.40	212.00	0.47	1.30
F ₂ -8	100.00	196.40	35.84	1.52	7.60	10.22	12.28	9.00	28.90	165.00	0.21	0.62
F ₂ -9	87.00	207.40	42.88	1.64	6.40	11.16	12.24	9.80	33.00	35.00	0.22	0.82
F ₂ -10	88.00	169.40	40.72	1.60	7.80	9.28	14.38	8.00	26.20	196.00	0.19	0.59
F ₂ -11	96.00	168.00	38.40	1.00	7.20	8.01	14.50	8.60	21.00	205.00	0.27	0.49
F ₂ -12	87.00	207.00	44.64	2.32	5.80	13.27	13.12	9.60	30.20	301.00	0.26	0.79
F ₂ -13	94.00	162.20	38.08	1.60	9.00	8.14	11.74	8.60	22.76	162.00	0.26	0.52
F ₂ -14	81.00	234.60	44.14	1.74	7.80	12.97	12.90	9.20	30.80	112.00	0.39	1.27
F ₂ -15	85.00	200.20	45.64	1.88	7.00	11.08	16.72	8.80	33.00	106.00	0.19	0.76
F ₂ -16	87.00	177.40	47.06	1.58	5.60	9.97	15.48	7.80	30.40	216.00	0.11	0.35
F ₂ -17	99.00	211.80	44.32	2.04	4.60	9.53	12.54	10.40	29.90	161.00	0.18	0.40
F ₂ -18	97.00	189.60	34.30	1.94	8.80	12.12	12.88	10.80	31.24	93.00	0.34	1.08
F ₂ -19	85.00	170.20	43.50	2.10	8.80	11.52	13.02	8.20	30.00	176.00	0.24	0.83
F ₂ -20	85.00	214.80	37.98	1.90	5.00	9.40	16.00	7.60	26.60	172.00	0.16	0.33

F ₂ -21	91.00	144.40	37.08	1.34	8.60	6.98	13.98	6.80	23.60	111.00	0.16	0.41
F ₂ -22	100.00	177.60	36.38	1.66	4.00	9.48	11.24	10.80	25.70	139.00	0.09	0.27
F ₂ -23	81.00	199.00	37.46	1.70	8.80	9.44	14.92	8.40	31.00	170.00	0.36	0.62
F ₂ -24	90.00	222.80	47.02	2.08	8.00	12.68	12.84	9.80	37.60	203.00	0.47	1.08
F ₂ -25	99.00	215.60	51.02	2.12	6.40	14.32	12.08	11.20	31.78	137.00	0.37	1.11
F ₂ -26	92.00	212.60	41.82	2.00	10.00	11.45	12.72	10.00	31.40	341.00	0.30	1.02
F ₂ -27	100.00	188.60	41.58	1.72	4.60	8.73	12.68	7.40	24.34	110.00	0.10	0.30
F ₂ -28	91.00	214.80	38.78	2.02	7.80	11.57	13.22	9.40	25.50	63.00	0.29	1.01
F ₂ -29	93.00	269.20	57.88	2.58	9.60	17.36	13.58	10.80	38.20	94.00	0.93	2.60
F ₂ -30	90.00	170.40	43.66	1.90	6.00	10.85	18.42	8.40	27.30	230.00	0.10	0.71
F ₂ -31	93.00	202.40	42.60	1.90	10.80	10.36	9.90	8.60	30.60	102.00	0.49	1.23
F ₂ -32	91.00	201.40	44.02	1.54	8.40	10.79	10.72	7.80	34.60	120.00	0.42	0.67
F ₂ -33	88.00	191.20	38.62	1.86	12.00	10.63	12.00	8.60	26.40	204.00	0.43	1.09
F ₂ -34	87.00	285.20	47.66	2.62	11.60	19.15	15.14	13.40	38.20	157.00	1.64	3.56
F ₂ -35	90.00	213.60	45.14	1.94	9.80	12.03	13.30	9.60	35.40	42.00	0.84	1.16
F ₂ -36	94.00	170.80	46.32	1.92	11.80	12.54	10.40	8.60	26.00	336.00	0.43	1.19
F ₂ -37	92.00	224.60	42.02	1.82	6.20	12.33	11.80	8.40	33.00	89.00	0.37	0.68
F ₂ -38	90.00	195.80	47.60	2.10	8.40	11.79	11.10	9.00	27.80	164.00	0.39	0.91
F ₂ -39	100.00	221.80	42.58	2.00	8.60	12.30	11.84	11.00	29.48	417.00	0.37	1.02
F ₂ -40	91.00	217.20	42.32	2.04	9.60	14.44	11.86	10.20	29.00	116.00	0.67	1.37
F ₂ -41	87.00	205.00	41.74	1.64	10.80	9.75	12.82	7.00	31.10	253.00	0.36	1.00
F ₂ -42	91.00	232.00	45.52	1.96	6.60	12.44	13.50	10.20	31.70	170.00	0.38	0.98
F ₂ -43	88.00	241.60	50.46	2.10	8.20	14.54	11.62	9.20	33.20	272.00	0.64	1.42
F ₂ -44	90.00	215.70	42.74	1.68	5.20	12.27	10.08	9.40	29.80	36.00	0.22	0.58
F ₂ -45	85.00	236.40	37.32	1.60	5.00	13.24	14.38	8.20	32.60	208.00	0.24	0.74
F ₂ -46	89.00	182.40	37.48	1.60	11.60	9.47	15.04	7.60	32.80	121.00	0.30	0.82
F ₂ -47	87.00	210.80	41.28	1.58	4.40	11.66	13.98	8.40	31.40	93.00	0.13	0.52

F ₂ -48	104.00	154.60	36.14	1.60	6.80	7.92	9.64	7.60	25.30	137.00	0.11	0.27
F ₂ -49	87.00	201.80	30.68	1.32	7.80	10.88	12.02	7.20	29.10	22.00	0.34	0.60
F ₂ -50	85.00	237.60	43.04	2.10	8.40	13.44	11.96	9.60	34.80	190.00	0.45	1.32
F ₂ -51	91.00	194.60	34.80	1.52	10.20	8.36	10.46	9.20	34.30	120.00	0.44	0.85
F ₂ -52	94.00	205.40	41.38	1.62	8.00	11.89	10.26	8.60	31.60	165.00	0.48	0.81
F ₂ -53	95.00	167.20	37.76	1.60	5.80	10.25	12.10	7.00	32.70	281.00	0.13	0.39
F ₂ -54	94.00	203.80	43.44	1.80	7.60	12.84	12.36	10.40	29.50	100.00	0.38	0.84
F ₂ -55	93.00	231.20	34.64	1.38	7.00	10.24	12.70	8.20	33.70	107.00	0.29	0.60
F ₂ -56	95.00	193.20	34.84	1.38	11.00	9.94	12.46	8.00	32.80	207.00	0.38	0.76
F ₂ -57	91.00	202.60	42.34	1.92	6.40	11.03	12.28	8.60	31.50	126.00	0.20	0.62
F ₂ -58	89.00	147.00	36.34	1.26	10.00	7.36	9.60	7.20	24.50	125.00	0.14	0.32
F ₂ -59	102.00	178.20	40.44	1.46	3.00	10.10	10.02	8.60	23.02	122.00	0.06	0.16
F ₂ -60	93.00	201.80	44.94	1.58	5.20	12.11	14.10	8.40	31.70	109.00	0.21	0.58
F ₂ -61	90.00	229.80	46.96	2.08	16.60	10.72	13.66	9.20	31.80	148.00	0.76	1.98
F ₂ -62	86.00	182.80	44.26	1.78	6.20	12.07	12.20	8.80	30.50	49.00	0.19	0.61
F ₂ -63	92.00	210.40	36.90	1.62	7.00	11.91	11.76	9.00	29.30	138.00	0.21	0.83
F ₂ -64	87.00	193.20	43.14	1.78	8.20	10.70	12.66	8.00	33.40	163.00	0.26	0.78
F ₂ -65	85.00	182.20	39.06	1.80	6.40	10.00	12.88	8.00	30.90	280.00	0.24	0.41
F ₂ -66	89.00	192.40	42.30	1.58	12.80	9.59	14.08	7.80	31.10	156.00	0.53	0.97
F ₂ -67	93.00	185.00	36.94	1.30	3.00	10.34	12.28	8.60	24.24	54.00	0.10	0.31
F ₂ -68	93.00	191.80	40.28	1.36	5.00	8.51	9.06	8.60	30.00	119.00	0.14	0.35
F ₂ -69	90.00	169.00	39.22	1.68	6.00	8.74	14.58	7.60	31.70	136.00	0.11	0.47
F ₂ -70	87.00	195.60	36.12	1.64	8.00	9.46	12.42	8.20	27.60	302.00	0.28	0.62
F ₂ -71	85.00	154.00	42.22	1.44	13.80	9.13	10.78	7.80	25.00	236.00	0.33	0.65
F ₂ -72	89.00	173.40	37.72	1.64	3.80	10.49	11.10	7.60	26.90	180.00	0.08	0.29
F ₂ -73	91.00	187.00	39.38	1.64	6.00	10.63	12.58	8.60	31.30	149.00	0.20	0.62
F ₂ -74	93.00	154.60	34.18	1.16	4.40	10.19	14.74	5.80	25.40	176.00	0.04	0.25

F ₂ -75	94.00	167.60	36.82	1.82	5.40	10.10	12.14	6.80	28.00	101.00	0.09	0.39
F ₂ -76	89.00	127.20	29.46	1.30	8.40	8.14	11.82	6.40	22.60	94.00	0.13	0.29
F ₂ -77	94.00	199.40	30.40	1.58	5.20	11.94	12.04	8.20	33.10	161.00	0.24	0.52
F ₂ -78	104.00	201.00	34.28	1.18	7.00	12.38	13.76	8.80	26.78	173.00	0.32	0.70
F ₂ -79	95.00	188.20	34.08	1.32	7.20	9.39	12.44	7.60	29.10	113.00	0.19	0.53
F ₂ -80	91.00	187.60	40.64	1.86	2.60	11.55	12.54	8.20	27.60	167.00	0.09	0.31
F ₂ -81	104.00	222.20	40.78	1.74	6.00	10.74	9.22	11.00	34.00	183.00	0.25	0.59
F ₂ -82	102.00	187.20	39.16	1.78	3.40	9.30	12.38	7.60	29.80	122.00	0.08	0.24
F ₂ -83	89.00	198.20	35.08	1.62	7.60	10.99	11.28	8.60	33.10	157.00	0.33	0.71
F ₂ -84	102.00	204.60	48.78	2.12	8.00	12.93	11.34	8.80	24.00	154.00	0.42	0.98
F ₂ -85	94.00	203.40	37.42	1.46	4.60	11.03	12.14	7.60	32.90	186.00	0.17	0.42
F ₂ -86	104.00	215.40	34.70	1.68	4.80	13.12	14.18	7.00	31.24	193.00	0.12	0.34
F ₂ -87	91.00	191.40	44.38	1.82	4.60	10.92	13.22	8.60	28.60	121.00	0.19	0.49
F ₂ -88	104.00	154.00	36.48	1.60	7.20	7.39	13.32	7.40	25.90	58.00	0.11	0.30
F ₂ -89	90.00	221.60	42.90	1.60	4.40	12.23	12.52	9.40	32.10	135.00	0.17	0.54
F ₂ -90	87.00	206.40	37.14	1.66	9.00	11.62	13.34	8.80	30.40	109.00	0.32	0.80
F ₂ -91	92.00	167.60	41.46	1.78	7.00	9.75	15.16	8.60	34.40	43.00	0.27	0.65
F ₂ -92	86.00	157.00	40.12	1.44	3.80	9.78	11.76	7.00	31.50	71.00	0.08	0.21
F ₂ -93	90.00	177.80	38.74	1.52	8.00	10.35	11.86	7.40	31.70	272.00	0.14	0.44
F ₂ -94	88.00	226.60	47.56	2.30	9.20	16.70	11.02	10.40	32.10	123.00	0.60	1.71
F ₂ -95	93.00	164.40	35.04	1.48	6.40	10.24	11.24	7.60	26.20	176.00	0.16	0.44
F ₂ -96	91.00	185.00	45.36	2.02	9.20	9.84	10.66	7.00	34.80	232.00	0.40	0.77
F ₂ -97	88.00	192.20	44.96	1.72	8.60	10.67	12.66	7.80	25.90	108.00	0.39	0.95
F ₂ -98	105.00	185.20	46.54	1.70	6.60	9.73	11.82	9.80	27.90	69.00	0.27	0.60
F ₂ -99	86.00	160.80	50.78	1.66	9.60	9.44	10.10	7.80	30.10	121.00	0.28	0.62
F ₂ -100	85.00	209.20	51.62	2.22	5.60	11.58	13.10	8.60	35.00	423.00	0.26	0.67
F ₂ -101	90.00	241.20	44.60	2.20	10.80	10.69	12.88	8.00	35.30	221.00	0.61	1.05

F ₂ -102	89.00	216.40	48.50	2.62	6.80	14.58	11.96	9.80	32.50	137.00	0.63	1.07
F ₂ -103	103.00	150.00	36.36	1.76	5.00	8.60	11.62	6.80	24.70	249.00	0.09	0.28
F ₂ -104	93.00	237.20	44.54	1.90	12.00	13.43	10.62	9.20	35.70	101.00	1.07	1.66
F ₂ -105	88.00	195.80	42.04	1.82	8.60	11.18	13.18	7.80	29.20	197.00	0.31	0.74
F ₂ -106	91.00	174.80	45.56	2.00	14.20	8.65	11.68	8.00	30.10	54.00	0.62	0.86
F ₂ -107	90.00	208.60	41.70	2.12	3.80	12.58	11.92	9.00	33.90	109.00	0.28	0.56
F ₂ -108	93.00	171.60	33.66	1.74	6.20	9.03	11.52	6.80	29.10	273.00	0.23	0.34
F ₂ -109	91.00	215.00	43.86	1.78	7.80	11.47	11.40	8.00	26.75	198.00	0.27	0.57
F ₂ -110	95.00	226.40	42.70	1.86	8.40	12.82	13.76	9.40	29.30	101.00	0.40	1.16
F ₂ -111	103.00	207.80	44.36	1.94	4.80	11.76	11.60	7.20	28.80	114.00	0.15	0.48
F ₂ -112	89.00	262.40	45.96	2.84	6.40	16.35	11.00	10.40	35.10	134.00	0.93	1.90
F ₂ -113	92.00	151.00	35.62	1.54	7.80	8.41	10.48	7.00	27.80	130.00	0.22	0.37
F ₂ -114	87.00	191.40	42.22	2.02	10.20	11.84	10.42	7.80	28.90	117.00	0.40	0.81
F ₂ -115	85.00	216.60	46.04	1.86	8.20	12.81	13.94	7.60	34.50	144.00	0.37	0.81
F ₂ -116	87.00	222.40	44.42	1.90	9.60	13.47	11.66	8.20	28.80	182.00	0.84	1.32
F ₂ -117	90.00	187.80	48.16	2.06	6.00	12.69	11.84	7.80	33.20	274.00	0.31	0.73
F ₂ -118	93.00	158.40	32.40	1.24	10.00	10.31	14.46	6.80	32.10	182.00	0.38	1.00
F ₂ -119	91.00	164.80	36.28	1.56	4.60	10.92	14.96	7.00	31.90	102.00	0.19	0.32
F ₂ -120	94.00	187.20	35.58	1.56	4.40	11.07	14.94	7.20	29.10	140.00	0.20	0.33
F ₂ -121	103.00	203.80	49.46	2.04	12.00	11.95	12.26	8.40	31.70	262.00	0.60	1.64
F ₂ -122	93.00	217.60	44.56	2.02	5.20	14.13	11.80	10.40	31.30	73.00	0.34	0.81
F ₂ -123	95.00	173.80	41.16	1.72	4.80	9.56	13.80	6.80	28.40	62.00	0.13	0.34
F ₂ -124	94.00	174.20	35.00	1.74	5.40	11.50	11.22	6.80	30.10	210.00	0.12	0.52
F ₂ -125	103.00	179.00	30.28	1.40	3.20	7.56	10.74	10.40	30.06	133.00	0.10	0.27
F ₂ -126	91.00	180.80	40.46	1.66	12.60	9.59	11.96	7.20	31.20	179.00	0.39	0.88
F ₂ -127	89.00	170.80	38.58	1.34	5.80	8.94	11.74	7.20	32.00	88.00	0.12	0.29
F ₂ -128	94.00	143.20	55.28	1.68	6.60	9.89	10.56	6.80	26.70	232.00	0.14	0.44

F ₂ -129	87.00	162.40	46.76	1.96	9.40	11.54	11.18	7.40	31.70	103.00	0.33	0.79
F ₂ -130	86.00	166.60	39.88	1.48	6.80	9.86	10.36	8.00	29.80	103.00	0.18	0.48
F ₂ -131	85.00	231.60	55.56	2.80	10.80	15.60	8.30	10.40	35.90	58.00	1.06	2.05
F ₂ -132	87.00	142.60	36.66	1.38	4.60	7.32	13.22	6.60	27.00	244.00	0.07	0.22
F ₂ -133	90.00	180.60	44.66	2.04	8.40	10.07	13.40	7.60	28.76	190.00	0.27	0.71
F ₂ -134	99.00	133.20	26.76	1.42	4.80	7.64	11.72	6.20	24.52	122.00	0.16	0.42
F ₂ -135	89.00	191.60	36.32	1.56	9.40	10.22	10.88	7.80	32.98	13.00	0.29	0.67
F ₂ -136	90.00	202.60	48.52	2.06	10.40	11.16	11.16	7.00	29.68	215.00	0.32	1.02
F ₂ -137	89.00	187.20	46.96	2.26	5.00	11.72	11.44	9.40	34.16	112.00	0.28	0.57
F ₂ -138	92.00	133.80	45.66	1.90	7.80	9.21	10.12	7.40	25.50	126.00	0.22	0.35
F ₂ -139	94.00	163.00	39.34	1.66	4.20	8.63	9.20	6.80	30.14	159.00	0.06	0.22
F ₂ -140	104.00	184.40	32.30	1.28	4.40	10.54	12.82	9.40	31.86	80.00	0.24	0.50
F ₂ -141	93.00	183.60	48.56	1.72	9.80	11.54	14.16	7.80	33.76	231.00	0.25	0.63
F ₂ -142	102.00	154.60	32.26	1.20	3.40	7.31	11.58	8.80	26.04	221.00	0.12	0.27
F ₂ -143	92.00	166.00	48.46	1.58	4.40	9.20	11.92	7.20	27.68	109.00	0.12	0.28
F ₂ -144	92.00	160.20	36.38	1.58	7.20	9.44	11.64	7.00	20.96	231.00	0.16	0.37
F ₂ -145	93.00	192.00	46.48	1.94	7.00	11.94	9.92	7.40	31.54	109.00	0.28	0.66
F ₂ -146	103.00	166.00	35.24	1.52	5.40	8.07	12.36	7.20	24.80	132.00	0.12	0.31
F ₂ -147	104.00	203.00	43.60	1.68	3.20	9.45	11.56	9.40	29.78	163.00	0.10	0.28
F ₂ -148	90.00	169.20	38.50	1.78	8.80	8.62	10.62	7.40	33.30	104.00	0.31	0.51
F ₂ -149	88.00	199.80	45.76	1.76	7.00	10.87	12.44	7.80	31.36	101.00	0.22	0.66
F ₂ -150	87.00	170.40	37.08	1.56	8.00	8.68	12.52	7.60	26.20	112.00	0.12	0.53
F ₂ -151	98.00	179.60	33.70	1.14	6.60	12.32	12.36	7.40	26.96	190.00	0.17	0.37
F ₂ -152	90.00	181.60	42.34	2.02	5.00	13.10	9.94	8.80	32.90	122.00	0.21	0.49
F ₂ -153	88.00	212.60	48.52	2.12	6.00	11.79	11.46	9.40	31.48	199.00	0.26	0.63
F ₂ -154	87.00	215.60	39.94	1.82	9.20	13.45	12.42	8.60	30.68	103.00	0.35	0.79
F ₂ -155	89.00	196.40	46.04	1.62	6.20	11.16	9.54	8.40	30.16	123.00	0.17	0.59

F ₂ -156	102.00	217.60	46.10	1.74	10.80	9.29	11.52	9.20	28.30	100.00	0.43	0.83
F ₂ -157	92.00	173.60	41.00	1.86	5.00	10.44	12.46	7.20	28.06	45.00	0.17	0.42
F ₂ -158	92.00	163.20	39.98	1.68	4.20	9.47	11.58	7.40	25.80	303.00	0.11	0.24
F ₂ -159	88.00	185.60	42.26	1.84	6.40	11.13	12.74	8.00	24.72	112.00	0.22	0.48
F ₂ -160	92.00	146.80	42.22	1.64	5.20	9.37	12.54	6.20	28.40	102.00	0.14	0.26
F ₂ -161	90.00	177.00	33.56	1.36	12.00	12.94	12.76	7.00	32.36	118.00	0.29	0.68
F ₂ -162	88.00	184.80	47.70	1.92	5.80	10.80	12.22	9.00	34.40	122.00	0.22	0.55
F ₂ -163	92.00	181.20	37.92	1.78	5.40	9.52	14.66	7.80	27.68	172.00	0.20	0.41
F ₂ -164	103.00	183.00	36.78	1.80	7.60	10.13	12.32	9.00	23.30	189.00	0.24	0.58
F ₂ -165	92.00	176.80	43.72	1.92	6.40	9.81	12.04	9.00	26.54	109.00	0.27	0.65
F ₂ -166	92.00	147.80	37.96	1.56	6.40	8.14	11.82	6.40	26.74	105.00	0.10	0.24
F ₂ -167	88.00	159.60	42.26	1.68	7.40	9.43	12.32	7.40	28.14	87.00	0.22	0.48
F ₂ -168	89.00	148.00	38.52	1.34	6.80	7.95	11.82	7.20	24.60	136.00	0.13	0.24
F ₂ -169	105.00	181.60	39.60	1.72	4.80	7.80	11.92	7.60	28.46	117.00	0.10	0.28
F ₂ -170	104.00	193.40	43.74	1.80	4.80	10.28	11.66	8.20	33.56	50.00	0.13	0.37
F ₂ -171	92.00	181.80	38.00	1.26	5.00	12.12	11.30	7.20	25.28	107.00	0.17	0.38
F ₂ -172	90.00	138.00	46.20	1.72	4.00	8.51	11.28	7.00	26.04	238.00	0.06	0.21
F ₂ -173	102.00	126.80	28.66	1.24	5.40	6.71	10.36	5.80	19.82	309.00	0.06	0.32
F ₂ -174	104.00	160.00	37.00	1.78	8.20	10.46	10.80	9.00	25.26	165.00	0.26	0.67
F ₂ -175	89.00	144.80	41.92	1.38	5.60	7.58	9.28	5.20	22.02	111.00	0.07	0.17
F ₂ -176	101.00	239.20	43.42	2.18	10.00	10.56	10.48	8.40	32.14	166.00	0.41	1.05
F ₂ -177	103.00	149.60	38.76	1.70	3.20	6.63	13.52	5.80	27.30	146.00	0.02	0.11
F ₂ -178	103.00	189.00	42.88	1.86	3.60	8.01	12.36	8.20	28.88	223.00	0.10	0.21
F ₂ -179	92.00	181.80	40.62	1.72	5.40	10.02	11.98	6.80	26.68	193.00	0.13	0.34
F ₂ -180	93.00	128.40	40.50	1.72	5.00	7.77	10.78	6.40	23.42	105.00	0.11	0.27
F ₂ -181	104.00	162.60	39.52	1.80	6.60	7.78	11.30	6.60	28.60	211.00	0.16	0.38
F ₂ -182	91.00	174.40	48.82	1.84	5.40	11.43	10.82	7.00	35.16	121.00	0.15	0.54

F ₂ -183	104.00	175.60	35.68	1.94	5.80	10.30	11.20	8.00	25.94	332.00	0.13	0.39
F ₂ -184	103.00	162.00	35.72	1.76	7.20	8.55	12.64	7.60	24.94	121.00	0.14	0.41
F ₂ -185	104.00	234.80	47.02	1.96	6.20	12.64	10.28	13.40	28.30	152.00	0.37	0.87
F ₂ -186	105.00	134.40	31.52	1.40	4.40	8.00	10.22	7.80	24.64	118.00	0.06	0.17
F ₂ -187	105.00	152.00	36.64	1.66	5.40	7.79	14.22	7.20	27.58	58.00	0.06	0.22
F ₂ -188	105.00	161.60	37.96	1.58	6.20	8.07	12.86	6.20	29.58	20.00	0.11	0.25
F ₂ -189	89.00	199.60	37.94	1.66	7.00	10.95	12.38	7.80	31.42	21.00	0.22	0.55
F ₂ -190	92.00	172.20	39.60	1.66	6.60	10.33	11.86	7.20	30.76	158.00	0.18	0.38
F ₂ -191	104.00	134.00	31.90	1.46	9.20	7.30	11.56	7.60	25.88	128.00	0.14	0.31
F ₂ -192	92.00	192.60	33.22	1.40	8.00	10.10	11.78	7.80	32.52	102.00	0.22	0.59
F ₂ -193	92.00	177.20	37.84	1.46	6.20	11.21	11.44	8.00	26.18	193.00	0.24	0.39
F ₂ -194	91.00	189.60	44.96	1.72	9.60	9.75	12.06	8.40	28.84	196.00	0.34	0.82
F ₂ -195	94.00	155.40	36.88	1.40	10.00	8.05	12.04	7.80	23.50	87.00	0.24	0.46
F ₂ -196	93.00	146.20	31.08	1.50	4.60	7.50	11.58	6.20	27.68	112.00	0.08	0.15
F ₂ -197	104.00	146.60	26.80	1.44	5.60	10.76	13.76	7.60	25.28	123.00	0.16	0.36
F ₂ -198	104.00	180.00	28.06	1.26	3.00	8.74	8.50	13.40	21.72	128.00	0.06	0.11
F ₂ -199	102.00	147.80	31.82	1.30	4.60	6.57	12.64	6.60	27.14	199.00	0.07	0.16
F ₂ -200	94.00	153.20	40.00	1.64	9.20	9.40	9.58	7.40	28.80	36.00	0.28	0.49
F ₂ -201	104.00	180.00	40.84	1.06	6.80	7.57	8.24	8.80	28.80	141.00	0.19	0.50
F ₂ -202	91.00	185.80	40.76	1.86	8.20	9.69	14.52	7.80	31.12	171.00	0.24	0.49
F ₂ -203	93.00	147.60	37.02	1.72	6.80	9.61	11.92	6.60	26.68	116.00	0.18	0.34
F ₂ -204	104.00	181.20	34.84	1.44	6.80	11.39	13.96	5.60	26.54	101.00	0.14	0.40
F ₂ -205	104.00	191.60	41.72	1.66	7.80	10.31	13.34	8.80	28.36	102.00	0.44	0.49
F ₂ -206	93.00	199.80	44.36	1.80	11.00	12.59	12.52	7.60	30.28	165.00	0.36	1.07
F ₂ -207	94.00	132.40	31.06	1.70	4.20	7.77	11.62	6.40	28.46	138.00	0.06	0.16
F ₂ -208	104.00	192.00	34.08	1.28	4.60	10.42	10.42	7.20	23.26	100.00	0.15	0.35
F ₂ -209	92.00	156.00	42.74	1.78	9.80	10.46	12.72	7.20	26.36	143.00	0.23	0.62

F ₂ -210	104.00	212.00	45.00	1.98	7.40	12.98	14.14	8.80	31.64	203.00	0.22	0.48
F ₂ -211	91.00	175.40	44.48	1.90	8.00	11.86	10.10	7.80	29.02	141.00	0.23	0.61
F ₂ -212	104.00	186.00	37.78	2.10	3.60	11.94	12.86	7.80	30.02	136.00	0.12	0.30
F ₂ -213	104.00	171.80	35.88	1.50	11.20	9.85	10.24	6.60	31.90	31.00	0.29	0.63
F ₂ -214	93.00	190.60	42.20	1.78	7.00	9.46	14.28	7.40	30.66	103.00	0.23	0.54
F ₂ -215	91.00	212.00	45.52	2.18	10.80	16.50	12.04	10.80	32.98	140.00	0.81	2.14
F ₂ -216	94.00	205.60	47.10	1.96	3.60	10.22	12.50	7.80	32.16	109.00	0.13	0.34
F ₂ -217	104.00	194.20	34.62	1.32	7.40	13.35	12.76	6.40	27.18	213.00	0.18	0.50
F ₂ -218	94.00	188.20	49.90	1.88	7.80	10.69	13.84	7.80	34.32	86.00	0.31	0.61
F ₂ -219	95.00	159.60	35.12	1.52	12.00	8.49	13.04	7.20	30.32	157.00	0.33	0.70
F ₂ -220	104.00	170.60	28.60	1.22	4.40	8.30	11.37	10.80	27.10	211.00	0.11	0.20
F ₂ -221	94.00	149.80	37.96	1.96	5.80	9.86	13.60	6.60	27.04	145.00	0.10	0.35
F ₂ -222	91.00	187.80	45.70	2.16	5.80	11.16	12.46	7.80	32.66	550.00	0.18	0.43
F ₂ -223	93.00	175.00	49.10	2.10	5.80	9.54	11.26	7.40	29.82	107.00	0.15	0.42
F ₂ -224	94.00	170.40	39.82	1.64	7.20	9.36	12.48	7.00	29.74	138.00	0.12	0.49
F ₂ -225	104.00	155.40	32.90	1.54	9.20	9.48	12.86	7.40	22.14	281.00	0.24	0.52
F ₂ -226	105.00	137.80	33.84	1.14	6.40	8.95	13.96	7.80	18.80	17.00	0.25	0.79
F ₂ -227	106.00	114.00	23.46	0.96	5.60	11.33	9.58	7.60	22.00	100.00	0.29	0.84
F ₂ -228	104.00	167.00	31.26	1.22	5.20	8.62	11.34	7.40	27.60	126.00	0.20	0.79
F ₂ -229	108.00	170.80	41.14	1.10	5.20	10.71	9.20	8.80	21.60	161.00	0.08	0.22
F ₂ -230	104.00	128.00	33.52	1.26	9.40	9.21	12.56	9.40	26.84	77.00	0.27	0.59
F ₂ -231	95.00	153.40	46.90	1.48	5.00	9.20	10.70	8.00	30.20	35.00	0.14	0.35
F ₂ -232	104.00	180.20	41.88	1.12	4.40	12.43	13.82	7.40	24.98	90.00	0.11	0.25
F ₂ -233	93.00	151.00	42.84	1.58	11.20	8.19	10.68	7.00	24.94	169.00	0.21	0.59
F ₂ -234	94.00	167.40	39.30	1.80	4.40	9.95	10.80	8.80	29.84	110.00	0.15	0.37
F ₂ -235	93.00	149.20	36.38	1.64	7.20	8.57	12.64	6.60	26.30	176.00	0.10	0.32
F ₂ -236	93.00	169.20	53.02	1.98	9.20	11.00	13.02	7.40	31.48	70.00	0.34	0.77

F ₂ -237	106.00	118.00	31.78	0.98	7.80	10.90	12.82	7.40	29.04	106.00	0.14	0.33
F ₂ -238	106.00	121.00	21.68	1.30	9.20	11.48	11.76	9.40	22.00	136.00	0.27	0.59
F ₂ -239	94.00	170.00	36.14	1.52	7.40	8.70	12.88	8.00	26.26	187.00	0.19	0.48
F ₂ -240	106.00	205.00	36.32	1.14	8.20	10.73	12.40	8.20	22.34	141.00	0.44	1.29
F ₂ -241	93.00	174.60	42.50	1.90	7.20	11.41	12.26	6.80	32.28	250.00	0.14	0.42
F ₂ -242	93.00	176.80	43.16	1.86	6.00	8.39	13.02	7.20	27.86	160.00	0.12	0.36
F ₂ -243	92.00	166.20	46.82	2.28	8.40	10.08	13.26	8.20	28.86	107.00	0.24	0.68
F ₂ -244	106.00	179.60	39.18	1.18	4.80	11.01	12.16	7.60	24.24	42.00	0.21	0.49
F ₂ -245	106.00	197.20	33.54	1.14	7.20	11.05	10.77	8.60	23.06	117.00	0.21	0.46
F ₂ -246	95.00	166.40	43.54	2.00	5.60	9.87	11.64	7.20	29.70	109.00	0.14	0.38
F ₂ -247	106.00	165.80	32.64	1.38	6.00	10.02	12.40	12.40	20.72	101.00	0.22	0.46
F ₂ -248	108.00	181.80	33.68	1.08	7.60	10.61	12.22	10.20	22.68	121.00	0.24	0.58
F ₂ -249	108.00	159.80	37.88	0.86	10.00	9.56	12.34	11.00	19.70	33.00	0.43	1.02
F ₂ -250	104.00	146.40	33.98	1.36	10.00	9.68	10.72	8.80	21.24	78.00	0.16	0.64
TetraBajra (P1)	76.00	131.00	48.00	2.63	6.40	15.90	9.50	7.80	31.40	452.00	0.25	0.57
FD-475 (P2)	104.00	257.00	53.00	2.10	13.80	13.10	14.40	11.60	24.70	30.00	0.42	0.97

F₂ Lines	LDW_PP	SDW_PP	LSR	DMY_PP	GFY_PP	CHL	Oxalate	SL	SB	TSW
	(13)	(14)	(15)	(16)	(17)	(18)	(19)	(20)	(21)	(22)
F ₂ -1	0.20	0.80	0.26	1.00	6.15	30.48	1.10	2.30	1.20	2.19
F ₂ -2	0.16	0.60	0.27	0.76	9.67	30.84	1.60	2.50	1.40	1.06
F ₂ -3	0.12	0.45	0.26	0.57	6.46	34.72	1.30	2.00	1.40	2.55
F ₂ -4	0.03	0.09	0.34	0.12	5.63	34.98	0.70	3.00	1.60	1.50
F ₂ -5	0.07	0.33	0.20	0.40	7.94	45.18	2.10	2.60	1.50	1.73
F ₂ -6	0.12	0.51	0.24	0.64	5.65	41.42	1.20	2.60	1.10	1.71

F ₂ -7	0.12	0.39	0.29	0.51	9.69	27.90	0.90	2.20	1.50	1.42
F ₂ -8	0.07	0.34	0.21	0.41	5.61	35.84	1.20	2.30	1.00	1.43
F ₂ -9	0.07	0.22	0.32	0.29	7.61	28.32	1.20	2.00	1.10	3.86
F ₂ -10	0.06	0.20	0.32	0.26	7.71	30.20	2.20	2.40	1.60	1.31
F ₂ -11	0.13	0.16	0.82	0.29	3.68	35.64	1.40	2.30	1.60	3.65
F ₂ -12	0.07	0.25	0.28	0.32	8.59	44.32	0.90	2.30	1.20	1.32
F ₂ -13	0.17	0.27	0.63	0.43	7.70	35.16	1.90	2.50	1.40	1.72
F ₂ -14	0.09	0.36	0.24	0.45	9.46	28.96	1.00	2.50	1.00	1.77
F ₂ -15	0.04	0.25	0.18	0.29	6.13	32.74	1.90	2.80	1.30	1.94
F ₂ -16	0.03	0.10	0.34	0.13	3.29	31.34	1.30	2.80	1.40	1.66
F ₂ -17	0.06	0.20	0.28	0.25	5.07	37.80	2.10	2.10	0.90	1.87
F ₂ -18	0.11	0.41	0.26	0.52	8.37	25.98	1.50	2.80	1.40	1.96
F ₂ -19	0.06	0.46	0.13	0.52	10.74	27.80	0.70	2.40	1.30	1.37
F ₂ -20	0.03	0.10	0.27	0.13	4.70	44.54	0.80	2.20	1.10	0.84
F ₂ -21	0.05	0.12	0.44	0.17	3.98	30.92	0.90	2.60	1.60	2.62
F ₂ -22	0.03	0.12	0.25	0.15	8.07	38.10	0.90	2.40	1.50	1.84
F ₂ -23	0.09	0.19	0.45	0.28	9.35	33.46	1.30	2.60	1.30	2.34
F ₂ -24	0.11	0.31	0.35	0.42	7.71	35.34	0.90	2.60	1.30	3.76
F ₂ -25	0.11	0.58	0.19	0.69	9.82	31.28	1.10	2.80	1.30	3.05
F ₂ -26	0.10	0.29	0.33	0.39	5.63	39.74	1.50	2.60	1.60	4.14
F ₂ -27	0.03	0.13	0.22	0.16	5.16	31.40	1.60	2.90	1.40	1.66
F ₂ -28	0.08	0.28	0.29	0.36	4.17	43.60	0.60	2.00	1.00	1.88
F ₂ -29	0.20	0.82	0.24	1.02	6.67	28.38	0.80	2.40	1.40	2.57
F ₂ -30	0.04	0.27	0.16	0.31	7.63	28.02	1.10	3.00	1.00	1.68
F ₂ -31	0.13	0.34	0.37	0.46	7.32	37.56	1.80	3.00	1.40	2.83
F ₂ -32	0.09	0.19	0.49	0.29	7.04	39.30	2.30	2.50	1.40	1.81
F ₂ -33	0.11	0.37	0.29	0.48	9.04	36.36	1.50	2.60	1.50	1.70

F ₂ -34	0.41	1.55	0.27	1.97	10.60	32.16	0.70	2.60	1.40	2.99
F ₂ -35	0.19	0.33	0.57	0.52	7.33	25.48	2.40	2.30	1.30	1.59
F ₂ -36	0.10	0.39	0.25	0.49	6.09	33.28	0.80	2.70	1.50	1.83
F ₂ -37	0.08	0.23	0.35	0.31	6.83	33.90	0.40	2.30	1.00	1.67
F ₂ -38	0.09	0.26	0.37	0.35	5.56	37.80	1.90	2.00	1.20	1.90
F ₂ -39	0.12	0.57	0.21	0.69	10.27	25.70	0.60	2.70	1.00	1.86
F ₂ -40	0.15	0.56	0.28	0.71	9.14	28.70	1.30	2.40	1.40	0.85
F ₂ -41	0.10	0.29	0.34	0.39	5.39	36.54	1.40	3.00	1.80	4.77
F ₂ -42	0.10	0.29	0.32	0.39	6.43	40.56	1.30	2.90	1.30	3.65
F ₂ -43	0.15	0.52	0.29	0.68	9.72	30.94	2.00	2.20	1.00	1.13
F ₂ -44	0.06	0.22	0.26	0.28	7.97	25.12	0.80	2.70	1.60	0.83
F ₂ -45	0.06	0.26	0.24	0.33	5.76	25.96	1.30	2.60	1.40	4.32
F ₂ -46	0.07	0.25	0.30	0.32	5.79	35.30	1.50	2.70	1.30	2.86
F ₂ -47	0.03	0.17	0.20	0.20	5.86	35.16	1.20	3.00	1.40	2.12
F ₂ -48	0.04	0.13	0.29	0.17	7.78	25.42	0.70	2.50	1.30	2.14
F ₂ -49	0.09	0.20	0.46	0.30	7.98	34.16	0.70	2.50	1.40	1.69
F ₂ -50	0.12	0.49	0.24	0.61	8.99	25.78	2.30	2.40	1.40	1.94
F ₂ -51	0.11	0.28	0.41	0.40	5.21	39.14	1.40	3.00	1.10	3.01
F ₂ -52	0.10	0.26	0.39	0.36	6.61	43.42	1.00	2.50	1.40	1.64
F ₂ -53	0.03	0.12	0.25	0.15	6.32	39.58	1.50	2.80	0.90	1.63
F ₂ -54	0.10	0.27	0.36	0.36	6.31	40.78	2.40	2.90	1.60	2.12
F ₂ -55	0.06	0.20	0.33	0.26	6.64	31.82	1.90	2.90	1.20	1.70
F ₂ -56	0.09	0.29	0.31	0.37	7.14	37.46	2.60	2.90	0.90	3.96
F ₂ -57	0.06	0.23	0.25	0.28	8.26	32.00	1.00	2.90	0.90	3.30
F ₂ -58	0.04	0.09	0.48	0.14	6.76	30.88	1.20	2.10	1.10	1.21
F ₂ -59	0.01	0.05	0.32	0.06	6.56	24.48	1.40	2.30	1.30	3.19
F ₂ -60	0.05	0.18	0.26	0.23	5.67	33.06	2.00	2.80	1.00	1.63

F ₂ -61	0.19	0.65	0.29	0.84	14.46	26.08	0.40	2.00	1.60	2.20
F ₂ -62	0.04	0.21	0.20	0.25	9.00	34.52	1.70	2.90	1.50	4.17
F ₂ -63	0.06	0.25	0.22	0.31	7.65	38.84	1.10	2.30	1.30	1.51
F ₂ -64	0.06	0.26	0.23	0.32	11.92	26.06	2.10	2.30	1.20	2.80
F ₂ -65	0.06	0.31	0.18	0.36	8.76	32.94	1.10	2.70	1.00	1.37
F ₂ -66	0.13	0.30	0.44	0.44	6.44	36.94	1.70	2.40	1.20	1.65
F ₂ -67	0.02	0.09	0.26	0.12	8.31	33.28	1.50	2.20	0.90	1.52
F ₂ -68	0.03	0.10	0.28	0.13	5.53	35.14	1.20	2.60	1.40	1.72
F ₂ -69	0.03	0.16	0.19	0.19	5.69	24.20	1.20	2.40	1.40	1.31
F ₂ -70	0.07	0.20	0.34	0.26	6.10	44.62	0.80	2.90	0.90	2.37
F ₂ -71	0.09	0.18	0.53	0.27	8.54	27.12	1.90	2.20	1.10	1.79
F ₂ -72	0.02	0.08	0.31	0.10	6.40	34.14	1.10	2.30	1.20	1.33
F ₂ -73	0.05	0.18	0.26	0.23	6.32	39.44	1.30	2.50	1.30	1.51
F ₂ -74	0.02	0.07	0.28	0.09	7.19	43.22	1.30	2.40	0.90	1.73
F ₂ -75	0.04	0.11	0.33	0.15	4.34	33.54	1.50	2.20	1.30	2.08
F ₂ -76	0.03	0.07	0.36	0.10	4.18	33.16	0.80	2.20	1.60	3.88
F ₂ -77	0.06	0.16	0.36	0.21	7.62	36.96	1.10	2.10	1.40	3.41
F ₂ -78	0.08	0.21	0.36	0.29	8.94	26.36	1.30	2.40	1.00	1.47
F ₂ -79	0.04	0.15	0.28	0.20	7.85	28.74	1.10	3.00	0.90	1.77
F ₂ -80	0.02	0.10	0.21	0.12	5.67	33.20	1.60	2.10	1.00	1.90
F ₂ -81	0.07	0.32	0.22	0.39	5.57	34.50	1.30	2.60	1.20	1.81
F ₂ -82	0.02	0.12	0.20	0.14	6.62	45.68	1.70	2.10	1.60	1.58
F ₂ -83	0.08	0.20	0.40	0.28	6.13	32.30	1.10	2.90	1.30	1.28
F ₂ -84	0.12	0.56	0.22	0.68	7.97	25.84	1.40	2.90	1.40	1.84
F ₂ -85	0.04	0.11	0.38	0.15	6.07	41.56	1.40	2.70	1.60	1.00
F ₂ -86	0.03	0.17	0.20	0.20	5.25	30.78	1.40	2.50	1.30	1.86
F ₂ -87	0.05	0.17	0.28	0.22	8.44	28.46	1.10	2.80	1.60	2.61

F ₂ -88	0.03	0.15	0.23	0.18	4.37	26.36	1.10	2.10	1.50	1.69
F ₂ -89	0.05	0.15	0.30	0.20	7.83	33.18	1.60	2.70	1.30	1.66
F ₂ -90	0.07	0.26	0.27	0.33	6.50	33.80	1.50	2.00	1.00	0.92
F ₂ -91	0.06	0.16	0.38	0.22	6.69	35.76	1.10	2.20	1.30	1.68
F ₂ -92	0.02	0.06	0.32	0.08	4.90	29.70	1.80	2.60	1.20	0.72
F ₂ -93	0.04	0.14	0.28	0.18	7.24	30.20	1.60	2.80	1.50	1.78
F ₂ -94	0.14	0.62	0.22	0.75	6.88	30.98	1.50	2.60	1.50	1.78
F ₂ -95	0.04	0.13	0.30	0.17	6.73	30.78	3.20	2.50	1.10	1.65
F ₂ -96	0.09	0.25	0.37	0.34	4.47	34.18	1.60	3.00	1.40	0.87
F ₂ -97	0.09	0.22	0.38	0.31	8.29	27.32	1.10	2.30	1.60	2.15
F ₂ -98	0.08	0.32	0.25	0.40	8.52	28.04	1.10	2.60	1.60	1.57
F ₂ -99	0.08	0.15	0.51	0.23	7.43	28.74	1.60	2.00	0.90	1.84
F ₂ -100	0.06	0.24	0.25	0.30	9.23	32.72	1.70	2.20	1.50	1.79
F ₂ -101	0.13	0.37	0.34	0.49	5.84	26.10	1.50	2.00	1.10	1.77
F ₂ -102	0.13	0.49	0.26	0.61	8.05	40.44	0.90	2.70	1.00	1.55
F ₂ -103	0.02	0.13	0.18	0.16	8.58	30.92	1.20	2.10	0.90	1.76
F ₂ -104	0.20	0.62	0.32	0.83	6.76	30.84	2.50	2.00	1.00	2.16
F ₂ -105	0.07	0.26	0.28	0.33	6.40	30.66	0.80	2.40	1.60	1.68
F ₂ -106	0.12	0.23	0.55	0.35	5.65	28.54	0.70	2.80	1.60	2.21
F ₂ -107	0.06	0.20	0.29	0.26	4.46	38.30	0.90	2.50	1.40	1.16
F ₂ -108	0.04	0.08	0.56	0.12	4.53	44.10	2.20	2.20	1.20	1.64
F ₂ -109	0.07	0.18	0.37	0.24	7.68	26.32	2.00	2.40	1.40	3.23
F ₂ -110	0.08	0.43	0.19	0.52	7.62	34.82	1.40	2.70	1.40	2.75
F ₂ -111	0.04	0.25	0.18	0.29	6.94	39.38	0.60	2.80	1.50	3.68
F ₂ -112	0.20	0.65	0.31	0.85	7.38	33.18	0.60	2.50	1.20	4.10
F ₂ -113	0.04	0.08	0.52	0.13	6.20	34.60	1.20	2.90	0.90	2.04
F ₂ -114	0.13	0.23	0.58	0.37	6.13	39.98	1.90	2.60	1.50	1.71

F ₂ -115	0.08	0.32	0.23	0.40	6.23	34.22	1.30	2.20	1.10	1.96
F ₂ -116	0.16	0.33	0.48	0.48	6.88	52.18	0.50	2.90	0.90	1.75
F ₂ -117	0.06	0.24	0.25	0.30	10.22	32.40	1.30	2.90	1.30	1.25
F ₂ -118	0.07	0.24	0.30	0.32	6.07	47.56	2.30	2.70	1.40	1.64
F ₂ -119	0.03	0.07	0.48	0.11	7.55	36.30	1.60	2.00	1.30	1.35
F ₂ -120	0.04	0.11	0.33	0.15	4.80	39.90	1.40	2.50	1.00	1.22
F ₂ -121	0.17	0.82	0.21	0.99	8.64	23.60	0.70	2.70	1.10	2.26
F ₂ -122	0.07	0.21	0.34	0.28	12.07	37.94	1.40	2.60	1.30	1.68
F ₂ -123	0.02	0.09	0.27	0.12	8.70	29.94	1.60	2.30	1.20	1.47
F ₂ -124	0.03	0.12	0.22	0.14	4.92	41.18	1.00	2.20	1.20	1.93
F ₂ -125	0.03	0.10	0.28	0.12	5.00	26.04	1.40	3.00	1.40	2.37
F ₂ -126	0.08	0.21	0.39	0.29	6.18	43.16	0.90	2.30	1.20	2.13
F ₂ -127	0.03	0.08	0.36	0.11	5.45	38.40	1.20	2.50	1.60	2.25
F ₂ -128	0.03	0.11	0.27	0.14	8.60	34.74	2.50	2.40	1.30	1.88
F ₂ -129	0.07	0.20	0.34	0.27	8.84	26.88	1.50	3.00	1.50	1.67
F ₂ -130	0.04	0.10	0.39	0.14	4.96	35.70	1.20	2.70	1.50	1.50
F ₂ -131	0.21	0.56	0.38	0.77	6.07	27.66	0.10	2.70	1.30	2.05
F ₂ -132	0.01	0.05	0.30	0.06	8.81	36.24	1.20	2.00	1.50	1.65
F ₂ -133	0.07	0.26	0.28	0.33	7.89	36.30	0.20	2.80	1.40	0.62
F ₂ -134	0.01	0.08	0.14	0.10	6.07	27.48	1.10	2.50	1.20	1.85
F ₂ -135	0.05	0.37	0.13	0.42	6.31	25.16	1.50	2.30	1.20	1.77
F ₂ -136	0.07	0.36	0.20	0.43	7.41	41.08	2.80	2.90	1.30	3.44
F ₂ -137	0.07	0.17	0.38	0.24	8.69	34.58	1.60	2.40	1.50	2.57
F ₂ -138	0.06	0.09	0.64	0.14	8.02	25.22	0.60	2.20	1.30	1.41
F ₂ -139	0.02	0.06	0.26	0.07	4.21	33.72	1.90	2.30	1.40	1.69
F ₂ -140	0.06	0.15	0.38	0.21	5.15	33.68	1.80	2.00	1.10	0.87
F ₂ -141	0.07	0.18	0.37	0.25	7.51	36.78	0.90	2.60	1.30	1.68

F ₂ -142	0.02	0.06	0.30	0.08	5.95	35.60	1.20	2.80	1.30	1.62
F ₂ -143	0.03	0.09	0.29	0.12	7.39	36.66	1.20	2.20	1.10	1.65
F ₂ -144	0.04	0.11	0.34	0.15	6.25	26.26	1.90	2.70	1.30	1.66
F ₂ -145	0.06	0.21	0.28	0.27	5.75	28.06	2.10	2.60	1.00	1.65
F ₂ -146	0.03	0.17	0.20	0.21	3.72	24.14	0.60	2.50	1.30	2.55
F ₂ -147	0.03	0.16	0.20	0.20	5.02	34.04	2.60	3.00	1.50	3.08
F ₂ -148	0.07	0.17	0.39	0.24	9.35	34.42	0.70	2.20	1.40	1.36
F ₂ -149	0.05	0.22	0.24	0.27	6.29	26.82	1.10	2.90	1.40	1.66
F ₂ -150	0.04	0.22	0.16	0.26	4.91	36.96	1.80	2.80	1.10	1.82
F ₂ -151	0.04	0.11	0.35	0.15	8.23	27.54	0.60	2.10	1.50	2.72
F ₂ -152	0.05	0.17	0.30	0.22	7.17	27.96	1.50	3.00	0.90	1.77
F ₂ -153	0.06	0.25	0.25	0.32	9.80	33.92	0.50	2.00	1.40	1.84
F ₂ -154	0.09	0.28	0.31	0.37	7.66	37.94	2.10	2.40	1.50	3.68
F ₂ -155	0.05	0.19	0.24	0.24	5.10	35.78	1.90	2.60	1.40	1.30
F ₂ -156	0.14	0.44	0.32	0.58	8.91	28.10	2.00	2.20	1.10	2.24
F ₂ -157	0.04	0.15	0.27	0.19	3.61	36.74	1.50	2.00	1.10	1.43
F ₂ -158	0.03	0.07	0.35	0.10	3.76	43.76	0.30	2.40	1.10	1.64
F ₂ -159	0.05	0.14	0.36	0.19	5.23	37.70	1.10	2.90	1.50	2.31
F ₂ -160	0.03	0.07	0.41	0.10	4.28	38.64	1.40	2.10	1.10	1.68
F ₂ -161	0.07	0.19	0.39	0.27	6.68	34.24	0.60	2.60	1.20	1.17
F ₂ -162	0.05	0.19	0.27	0.24	7.36	36.22	1.80	2.60	1.30	2.91
F ₂ -163	0.03	0.13	0.20	0.16	4.18	27.26	1.80	2.50	1.00	1.95
F ₂ -164	0.08	0.33	0.25	0.41	6.90	24.88	1.10	2.80	1.20	1.78
F ₂ -165	0.06	0.20	0.29	0.26	4.47	33.62	2.20	2.30	1.40	1.16
F ₂ -166	0.03	0.07	0.41	0.10	7.47	25.24	0.60	2.10	1.10	1.76
F ₂ -167	0.05	0.15	0.36	0.20	6.29	32.52	1.60	2.50	1.60	0.97
F ₂ -168	0.03	0.07	0.41	0.10	4.41	32.10	1.40	2.80	1.50	2.50

F ₂ -169	0.03	0.15	0.21	0.18	6.83	28.70	1.30	2.30	0.90	1.32
F ₂ -170	0.04	0.20	0.21	0.25	3.13	29.90	1.80	2.70	1.50	1.89
F ₂ -171	0.04	0.11	0.36	0.15	2.61	46.58	0.60	2.60	0.90	1.74
F ₂ -172	0.02	0.05	0.38	0.06	5.35	33.68	1.10	2.80	1.10	1.79
F ₂ -173	0.02	0.05	0.48	0.07	4.85	34.90	1.20	2.70	1.30	1.18
F ₂ -174	0.07	0.39	0.18	0.46	5.65	26.78	1.20	2.10	1.40	1.67
F ₂ -175	0.02	0.04	0.35	0.06	2.74	32.26	1.80	2.90	1.50	1.85
F ₂ -176	0.12	0.56	0.22	0.69	7.38	33.10	0.70	2.90	1.00	1.91
F ₂ -177	0.01	0.05	0.15	0.06	6.28	27.90	1.40	2.50	1.00	1.72
F ₂ -178	0.03	0.11	0.27	0.14	7.38	35.40	1.20	2.50	1.40	1.48
F ₂ -179	0.04	0.13	0.31	0.17	4.95	29.20	1.20	2.40	1.60	1.03
F ₂ -180	0.03	0.09	0.36	0.12	2.50	35.72	2.10	2.60	1.00	2.31
F ₂ -181	0.04	0.13	0.31	0.17	9.98	39.54	0.90	2.70	1.00	1.31
F ₂ -182	0.05	0.21	0.22	0.25	5.10	34.80	1.40	2.20	1.10	1.27
F ₂ -183	0.04	0.23	0.18	0.27	4.15	26.34	0.90	2.80	1.60	1.28
F ₂ -184	0.04	0.22	0.18	0.26	5.93	34.54	0.90	2.10	1.30	1.78
F ₂ -185	0.13	0.51	0.26	0.64	3.59	31.28	1.30	2.80	1.60	1.20
F ₂ -186	0.02	0.09	0.21	0.11	6.18	27.14	1.70	2.70	1.50	4.36
F ₂ -187	0.02	0.13	0.17	0.15	6.90	26.56	2.10	2.60	1.10	1.24
F ₂ -188	0.02	0.13	0.17	0.15	6.26	33.20	1.50	2.20	1.60	1.79
F ₂ -189	0.06	0.18	0.35	0.25	4.24	27.56	1.30	2.50	1.60	2.85
F ₂ -190	0.05	0.12	0.41	0.16	5.22	25.50	1.80	2.90	1.40	3.57
F ₂ -191	0.04	0.16	0.23	0.20	6.71	27.16	2.30	2.30	1.10	1.03
F ₂ -192	0.07	0.19	0.37	0.26	9.64	27.92	1.20	2.90	1.60	4.66
F ₂ -193	0.06	0.15	0.38	0.21	8.02	29.40	1.30	2.50	0.90	1.09
F ₂ -194	0.11	0.26	0.42	0.36	8.37	25.12	1.70	2.20	1.00	1.78
F ₂ -195	0.08	0.16	0.48	0.24	11.11	31.32	1.40	2.90	1.60	2.05

F ₂ -196	0.03	0.05	0.58	0.08	6.98	25.76	2.30	2.60	1.00	1.76
F ₂ -197	0.04	0.11	0.36	0.15	7.61	28.82	2.50	2.30	1.00	1.64
F ₂ -198	0.01	0.03	0.41	0.05	4.38	27.48	3.10	2.90	1.40	1.89
F ₂ -199	0.02	0.07	0.28	0.09	7.45	36.38	1.50	2.30	1.60	1.69
F ₂ -200	0.08	0.14	0.55	0.22	6.19	36.92	1.40	2.00	1.30	1.96
F ₂ -201	0.02	0.11	0.15	0.12	8.96	26.68	2.10	2.10	1.50	1.93
F ₂ -202	0.07	0.16	0.44	0.23	10.82	39.18	2.10	2.80	1.00	1.40
F ₂ -203	0.06	0.10	0.55	0.16	9.26	32.78	2.20	2.30	1.10	1.77
F ₂ -204	0.02	0.11	0.14	0.13	9.75	47.26	3.30	2.20	1.30	1.73
F ₂ -205	0.08	0.31	0.25	0.39	11.67	40.14	1.70	2.00	0.90	1.75
F ₂ -206	0.09	0.40	0.22	0.49	10.93	49.16	1.80	2.60	1.40	1.68
F ₂ -207	0.02	0.06	0.34	0.08	4.23	28.28	1.20	2.00	1.00	1.70
F ₂ -208	0.05	0.20	0.25	0.25	6.94	33.76	1.70	2.60	1.50	1.12
F ₂ -209	0.05	0.19	0.27	0.24	10.52	35.08	1.90	2.10	1.20	1.68
F ₂ -210	0.05	0.15	0.36	0.20	8.16	35.54	2.30	2.00	1.00	1.86
F ₂ -211	0.05	0.22	0.25	0.27	11.25	36.88	1.70	2.10	1.00	1.14
F ₂ -212	0.03	0.11	0.27	0.13	7.04	29.02	1.70	2.70	1.00	1.93
F ₂ -213	0.07	0.19	0.35	0.26	6.25	39.08	0.80	2.60	1.30	1.67
F ₂ -214	0.06	0.18	0.32	0.24	13.85	29.60	1.60	2.20	1.00	1.77
F ₂ -215	0.19	0.76	0.24	0.95	8.24	26.66	1.50	2.50	1.00	1.89
F ₂ -216	0.03	0.12	0.28	0.15	6.93	32.18	1.10	2.40	1.60	1.77
F ₂ -217	0.06	0.17	0.33	0.23	12.57	26.74	2.10	2.10	0.90	1.68
F ₂ -218	0.08	0.20	0.39	0.28	9.03	31.26	1.60	3.00	1.30	1.80
F ₂ -219	0.07	0.21	0.35	0.28	5.16	41.80	1.40	2.60	1.10	1.73
F ₂ -220	0.03	0.07	0.48	0.10	10.61	27.92	1.40	2.40	1.50	1.39
F ₂ -221	0.03	0.11	0.24	0.13	7.56	39.96	1.80	2.30	1.40	1.83
F ₂ -222	0.05	0.15	0.33	0.19	6.12	27.18	1.10	2.30	1.40	1.79

F ₂ -223	0.04	0.13	0.33	0.18	5.26	39.04	2.10	2.50	1.30	1.99
F ₂ -224	0.03	0.25	0.11	0.28	8.36	36.20	1.40	2.60	1.60	1.73
F ₂ -225	0.06	0.16	0.35	0.21	6.68	45.34	0.60	2.50	1.50	1.64
F ₂ -226	0.08	0.30	0.26	0.38	6.63	28.20	1.70	2.50	1.50	1.48
F ₂ -227	0.07	0.27	0.27	0.34	8.55	32.12	1.70	2.30	1.30	1.56
F ₂ -228	0.06	0.24	0.26	0.30	7.95	33.84	1.70	2.30	1.00	1.85
F ₂ -229	0.01	0.06	0.21	0.07	9.72	37.20	2.20	2.60	1.10	1.89
F ₂ -230	0.06	0.19	0.29	0.25	6.73	40.18	1.40	3.00	1.20	0.83
F ₂ -231	0.03	0.10	0.28	0.13	9.72	43.00	2.50	2.40	1.60	1.48
F ₂ -232	0.03	0.08	0.35	0.10	10.31	35.24	1.40	2.00	1.10	1.53
F ₂ -233	0.07	0.16	0.43	0.22	6.72	27.44	2.60	3.00	0.90	0.97
F ₂ -234	0.04	0.11	0.39	0.15	8.25	28.42	1.30	2.70	1.50	1.81
F ₂ -235	0.04	0.08	0.50	0.12	9.01	31.74	2.50	2.10	1.40	1.67
F ₂ -236	0.07	0.20	0.36	0.26	5.92	32.60	1.10	2.50	1.60	1.22
F ₂ -237	0.03	0.14	0.22	0.17	8.50	44.36	1.60	2.90	1.10	1.79
F ₂ -238	0.07	0.18	0.36	0.25	10.10	30.40	2.10	2.70	1.00	1.35
F ₂ -239	0.04	0.12	0.35	0.16	5.52	31.04	1.90	3.00	1.00	1.35
F ₂ -240	0.11	0.48	0.24	0.59	7.83	32.42	1.10	2.50	1.10	1.64
F ₂ -241	0.03	0.11	0.31	0.15	6.64	26.50	1.40	2.80	1.30	1.72
F ₂ -242	0.03	0.10	0.28	0.12	7.21	31.58	1.80	2.80	1.30	1.12
F ₂ -243	0.05	0.20	0.25	0.26	9.85	26.98	1.10	2.30	1.60	1.67
F ₂ -244	0.04	0.15	0.29	0.20	7.38	33.60	2.70	2.70	1.00	1.67
F ₂ -245	0.05	0.14	0.36	0.20	4.59	28.42	1.60	2.40	1.20	1.29
F ₂ -246	0.04	0.13	0.33	0.17	8.38	37.40	2.30	2.50	1.20	1.95
F ₂ -247	0.03	0.15	0.20	0.18	8.82	36.94	2.30	2.30	1.60	1.87
F ₂ -248	0.08	0.33	0.25	0.41	7.03	27.84	1.90	2.40	1.40	1.90
F ₂ -249	0.09	0.32	0.29	0.41	5.38	33.86	1.50	2.50	1.10	1.89

F ₂ -250	0.04	0.11	0.41	0.15	11.27	36.18	2.40	2.00	1.50	0.80
TetraBajra (P1)	0.07	0.19	0.35	0.26	2.49	44.10	0.40	2.80	1.90	6.71
FD-475 (P2)	0.12	0.22	0.54	0.34	4.98	39.74	3.10	1.00	1.00	0.76

Appendix-II

List of polymorphic primers along with their sequences used for constructing framework linkage map in Bajra Napier Hybrid

S. No.	Primer Name	Forward Primer sequences	Reverse prime sequence	Source
1	CTM-10a	GAGGCAAAAGTGGAAGACAG	TTGATTCCCGGTTCTATCGA	Azevedo et al. 2012
2	PSMP2237	TGGCCTTGGCCTTTCCACGCTT	CAATCAGTCCGTAGTCCACACCCCA	
3	PSMP2274	CACCTAGACTCTACACAATGCAAC	AATATCAAGTGATCCACCTCCCAA	
4	PGIRD44	CACCTAGACTCTACACAATGCAAC	AATATCAAGTGATCCACCTCCCAA	
5	PGIRD49	AGCTCCTCGACGGAGAAAGT	GACGGTGTCGACGAAGATG	
6	PGIRD54	GCCTGGGATGTGTTTCTTCT	GCCTTTCATTTCCACCATGA	
7	CTM-8	GCTGCATCGGAGATAGGGAA	CTCAGCAAGCACGCTGCTCT	
8	CTM-10b	GAGGCAAAAGTGGAAGACAG	TTGATTCCCGGTTCTATCGA	
9	PSMP2248	TCTGTTTGTGTTGGGTCAGGTCCTTC	CGAATACGTATGGAGAAGTGC GCATC	
10	PSMP2255	CATCTAAACACAACCAATCTTGAAC	TGGCACTCTTAAATTGACGCAT	
11	PSMP226	CAAGGATGGCTGAAGGGCTATG	TTCCAGCCCACACCAGTAATC	
12	PGIRD5	CAACCCAACCCATTATACTTATCTG	GCAACTCTTGCCTTTCTTGG	
13	PGIRD25	CGGAGCTCCTATCATTCCAA	GCAAGCCACAAGCCTATCTC	
14	PGIRD56	ATCACTCCTCGATCGGTCAC	ACCAGACACACGTGCCAGT	
15	ICMP3001	GCCGTCGACATTAACCAACT	AAACATAGGGCTTGGCACTG	Kendel et al. 2016
16	ICMP3049	GAGCTGAACACGCTCAAGG	CAGATGACATCCATCCGTTG	
17	ICMP3063	TCCGGTAGAGACCGTAATGG	GGCACTCCCTAGCAAAATGA	
18	ICMP3095	GGGAGGCCACGATTTAAGA	ACAATGTGCACGCAAGGA	
19	PSMP708	GCAAGTAACCAACAATCCTTGA	ACCCCTAATACCCAAGCAATT	
20	ICMP3094	GACCTCGACCTCATCTCCAA	CGACAGCGAACTGGGATTAC	
21	ICMP3020	GTTCCATGGAGCTGGAAGTC	GCTAGAACAGGGCCGTTACA	
22	ICMP3021	GCCGACAGGAAGATTACGAT	AGCAAAACGCAGAACAACAG	
23	ICMP3033	GAGGGCCAGCTCTCCTAGAT	CCCTAACCACAGAGGGACAC	

24	ICMP3055	CCCAAACGCAAGTAGGGTTA	CCTTCTCCTGCCCCAGAC	
25	PSMP428	CCCGGTTGCAGTAGGTCTAG	AATACTCCATAATCGCCACTCC	
26	PSMP2090	AGCAGCCCAGTAATACCTCAGCTC	AGCCCTAGCGCACAAACACAAACTC	
27	PSMP2209	TTGGACGATTTGGAAGCATAG	GAGGAAAAGAGCCATACAGAGAC	
28	ICMP3002	CGAGCCGCCATAGTTGAC	TACACACACATTGCCACACG	
29	ICMP3028	ACGATTCTTCGTCGTTCCAG	GATACGCGCGAGCTACATTT	
30	ICMP3045	ACAAGGACGACAAGGACCAC	CCTCTCCAAGCACATGTTTC	
31	ICMP3056	ACGGAGCTACGGTTGGAATA	CACAAGGGACCCACGATA	
32	ICMP3068	CTGGCAAAGTTGTAGCGTGA	ATGTCGCTCTCTGCCAAGAT	
33	ICMP3069	TAGGAGGGGACTGCTCCTTT	AGGAAGAGGATGGTGGTGTG	
34	ICMP3081	ACGCCGTTTTTCGTGTAGTCT	TCCACAAGGTGACCTCACTG	
35	ICMP3096	CTGCATTGCAACATCCTCAC	AACCTGCAGTGAAGCAATC	
36	PSMP737	ATGCTTTTCCCCCGCTATCA	TCCTAGCAAGCTCGCATAAGA	
37	PSMP2019	TGTGCCACAGCTTGTTCTC	CAAGCAGCCAGTTCCTCATC	
38	PSMP2024	ACACCTTAGCAAAGGCTGCTAC	ATGCTTAGTTTGCTATAACTTGGG	
39	PSMP2222	TGGCTTCCAGACTAATCATCAC	TTATTTTAGCGGCGAGATTGAC	
40	ppssr0189	CCAACATCGCCCAATACTCT	CGTCGTTTCCTCGGTAAGAA	Lopez et al. (2018)
41	pssr0002	GTCTCGACAAACCCCAATGT	CAGTCCAGTGATGAAGCCTG	
42	ppssr0184	AGAGACACCACTGGGGATTG	AATTTGTTTCCAGCCCTCCT	
43	ppssr0189	CCAACATCGCCCAATACTCT	CGTCGTTTCCTCGGTAAGAA	
44	ppssr0293	TCTGAGTTGTTGCAGTTCGC	CTTTGCACCAACACGTTTAC	
45	pssr0366	CAGATCGCAGACAGGTACGA	CACCTAGGGCAGAGTAGGGTC	
46	ppssr0395	ATGTTTGTGGTGCACATGCT	CAAGCTTATGGGCACCCTAC	
47	ppssr0040	GGACACCGTTCTGCTTTTGT	CACAATGGCACATTCACACA	
48	ppssr0703	CTCCTGAGGTGCTGAGTGT	ACGTGGCCATTTTGTCTTAC	
49	ppssr0170	GCCACCAGCTCTTTTGCC	CAGAACTCACGATGCCAGAA	

50	ppssr0660	CGATATCGTCGTCAGCGTAA	GGGTTTCCCTTGCCTGTATT
51	ppssr0195	GTTCGACCAGAGAGCCTACG	TGGAAGGCATTGCCCTAGTA
52	ppssr0536	TCTCGTCTTGCTTTTGGAGG	TCCTACACCGGGACTTATGC
53	ppssr0090	TCCACGAGGACATCAACTCA	CCACCAATCTCTCAAGTCCA
54	ppssr0092	GCTTTTCCTACGAACCAGCA	CGTGTTGCTGTCCTAAACGA
55	ppssr0174	TCCAATGGTGTGTCACTCGT	GCGGTATCTAGTGGAGGCTG
56	ppssr0205	TGCTTGTGGTACAGCAAAGG	ACACTTTTTCGCCGAGTGTC
57	ppssr0279	GAAACAAAGAGATACAAGGAAGGA	GCAAGCGCTAAGTGGTCAAT
58	ppssr0440	TGGCACTCAAGATAAATGCG	CTTGTGGTATGACTCGCCCT
59	ppssr0529	ACCATATAAGGGAGGTGGGG	TTCGAACTCTAACAGGGAGATACT
60	ppssr0605	ATCTCACGAGGTGCCAAGAT	AGTCACGACCCCTCTTTTCC
61	ppssr0011	CTGATCTGTGAGGCAATGGA	CCAATTTTGGGTGCGAATAG
62	ppssr0242	TTCTGTACTGCTGCTCAGGC	AGAAGGACAAGGTTGGGAGC
63	ppssr0381	ACCCTTCCCAACCACTTTCT	CGAGGAAGGAACATCTCCTG
64	ppssr0435	AAGACACACACCCCAACC	GAGGAGAGGCGAAGAAGGAG
65	ppssr0006	ATCATATAGCCAGCAACGGG	CCCTCTTTCTCGTGCTATCG
66	ppssr0044	CATAAGCTTTCACCGTCCGT	CTCTGCACTGCCAAAATTGA
67	ppssr0058	GACCTCGGATTTCCCCAGT	GCCACCTGTTCAACAAAACA
68	ppssr0059	GCTTGTTTCCATCGCAAGAT	CTACCTGCTCAGCCACTGGT
69	ppssr0083	TCCGAGGAACACTTCTCCAG	CCCTTGATTGTTTGATGGCT
70	ppssr0224	TCAGAAGTGCTCCGAAGGTT	GCAGCGTCACCCCTATAAAA
71	ppssr0342	TGCTGAAGCAAAAGCAGGTA	TGGCAGCCAACCATAAATTC
72	ppssr0360	GCAGCTCGTGGATCTTCACT	GCGAGGGGACCAGATAAGTT
73	ppssr0364	AGCTAGCGCTGAGAGGGAG	CCTCCTACCCAAGTTCCACA
74	ppssr0397	TAGGTCAGAATGGACCCAGG	TAATCACACAGAACGCCAG
75	ppssr0547	GATCGGGTTGGTCTGTGTCT	GAGAACAGGAGGGGTGATGT

76	ppssr0590	GGCACCTAGAGACTCCACA	TCGGTGATGAAGTGAATTGC	
77	ppssr0628	AGAGCAAGGGTAAGAACCCC	CCCTCAGCCTTATCGTGCT	
78	ppssr0729	AAGTTCCTCAGGTCCCTCGT	TAGGCGAGCTCGTACCAGAG	Zhou et al. 2018
79	ppssr0176	GCTGGGTGTGGTGGTTAGTC	GCGAACGAGAGAAGAAAACG	
80	ppssr0215	TAGCCGGAATGAGAGTGCTT	GGTGACAGTGACGCAAAGAA	
81	ppssr0271	CTGCTGACGAGTGGCATTTA	GTGGAGGTAGGCTAGGAGGG	
82	PSSR3	GTCTCTGATGGCGTGGAACA	CTCCAAGATGGACAAGGCGT	
83	ppssr0328	TGCACGCAGGCTTATTATTG	CAGACCTGTGCGTGTAGTTCA	
84	PSSR22	CATTCAACCAGAGCCCACCC	GTCCTTCTTGCCCTCTTCC	
85	PSSR25	AAGCCAGAAACCCACGAAA	GACGCGTAGGATAGGGGTTG	
86	PSSR27	GCAAGCGATGGGAATGCAAA	GGCATATTCCCCAACGGTGA	
87	PSSR32	CTTCATGAGCAGCTCACGGT	CACGCCGGATTCTACCAAGA	
88	PSSR36	TGAAGATGAGAACGCGCTGA	CCATGAGTCCACATGTGCCT	
89	PSSR38	CCAAGCGCTCCTTCTTCTGA	CACGGCACTGATCACTCTGT	
90	PSSR41	GAGGGAGGGAAGGGGGAAG	GGACCGCTGAATTACCACCA	
91	PSSR44	ATCAAAGCTGATCTGGCCGG	GCGGCTCAACTCCATCTACA	
92	pssr0434	AAGAGGCACTATCTTATTGCTTGA	AGTGTGGTGTGCATGTTCGT	
93	CTM-12	GTTGCAAGCAGGAGTAGATCGA	CGCTCTGTAGGTTGAACTCCTT	
94	pssr0448	GGAGACGTGGATGGATCAAA	GACGGCGTAGACCGACTAAA	
95	pssr0582	GTCGCACATCATCACCAAAC	GGCAGTTGCACTTGCAGATA	
96	pssr0741	CACACTCCTGCACCAAGCTA	GTCAGCGGATCTCTCCACC	
97	ppssr0014	AGAAGGGGAGAAAAGGCAAA	AGCAGTGCAACGACACAAAC	
98	ppssr0053	GTATGCGATGCATGGGAAC	TATGCAAATCACTCCCCTCC	
99	ppssr0117	TCAAGCATCTTCGCAACAAC	AGTGTTATCAGGCTTGCGCT	
100	ppssr0162	TTGTCACGTGCAGAGCGGAG	TGCGACACACACTACACACC	

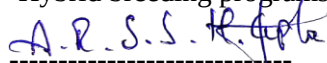
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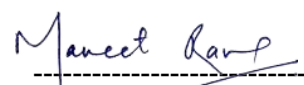
ABSTRACT

Bajra-Napier (BxN) hybrid is an interspecific manmade perennial grass also known as king's grass. It is very popular among dairy farmers and is also a potential bioenergy crop due to high biomass. It combines the unique features of both Bajra (*Cenchrus americanus*) and Napier (*Cenchrus purpureus*) species, which makes it more resilient to harsh environments with superior fodder quality. Genetic and genomic resources in BN Hybrid are very meagre due to non-availability of fertile seed producing hybrid till date. Recently, ICAR-IGFRI has developed the world's first fertile seed producing Bajra Napier Hybrid. The present study entitled "*Construction of framework linkage map and genetic dissection of biomass related traits in Bajra-Napier Hybrid*" was carried out during Kharif 2022 at Central Research Farm, Crop Improvement Division, ICAR-IGFRI, Jhansi. In current investigation, a total of 250 immortalized F₂ population were transplanted in Augmented Randomized Block Design (RCBD) for the construction of linkage map and phenotypic characterization was done for 22 biomass related traits. Genetic variability, heritability, genetic advance, correlation studies, Principal component analysis and cluster analysis were done in order to depict the variation in the population. A total of 217 markers were used to screen the parents for polymorphism. Out of these, 138 showed parental polymorphism and checked for validation in population with the help of randomly selected genotypes. 100 out of 138 were finally genotyped. The first-ever framework linkage map of Bajra Napier Hybrid was constructed with 88 markers loci. It has a stretch of 858.08 cM with an average marker density of 9.75 cM. The markers were unevenly distributed among the seven linkage groups. Analysis of variance revealed significant differences for all 22 measured traits. Significant effect of environment was also observed for the traits leaf length, leaf breadth and spike length. Phenotypic data from the immortalized F₂ lines were used to identify QTLs for biomass related traits by composite interval mapping (CIM). A total of 33 QTLs (LOD $\geq$ 3.0) were detected across 13 biomass related traits. Phenotypic variation explained (PVE) by QTLs ranged from 10.8 to 70.64 per cent. The highest phenotypic variation (70.64 %) was explained by the QTL for spike length.

In conclusion, it is envisaged that the present linkage map, fortified with 88 SSR markers and 33 QTLs for biomass related traits would provide genomics tools to breeders for further genetic enhancement of this crop species. Thus, the current study would serve as a strong foundation for further validation and fine mapping of QTLs for utilization in BN Hybrid breeding programs.



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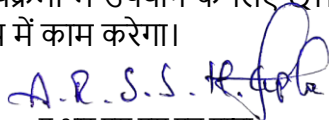
**रानी लक्ष्मी बाई केंद्रीय कृषि विश्वविद्यालय
कृषि महाविद्यालय, झाँसी
आनुवंशिकी और पादप प्रजनन विभाग**

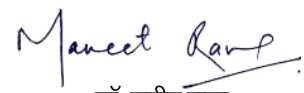
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डिग्री	: एमएससी कृषि
प्रमुख अनुशासन:	: आनुवंशिकी और पादप प्रजनन
थीसिस का शीर्षक:	: बाजरा-नेपियर हाइब्रिड में फ्रेमवर्क लिंकेज मानचित्र का निर्माण और बायोमास से संबंधित लक्षणों का आनुवंशिक विच्छेदन
प्रमुख सलाहकार:	: डॉ. मनीत राणा

अमूर्त

बाजरा-नेपियर (बीएन) संकर एक अंतरविशिष्ट मानव निर्मित बारहमासी घास है जिसे घास के राजा के रूप में भी जाना जाता है। यह डेयरी किसानों के बीच बहुत लोकप्रिय है और उच्च बायोमास के कारण एक संभावित जैव ऊर्जा फसल भी है। इसमें बाजरा (*सेक्रस अमेरिकेन्स*) और नेपियर (*सेक्रस परप्यूरियस*) दोनों प्रजातियों की अनूठी विशेषताओं का मिश्रण है, जो इसे बेहतर चारे की गुणवत्ता के साथ कठोर वातावरण के प्रति अधिक लचीला बनाता है। बीएन हाइब्रिड में आनुवंशिक और जीनोमिक संसाधन आज तक उपजाऊ बीज उत्पादक संकर की अनुपलब्धता के कारण बहुत कम हैं। हाल ही में, भारतीय कृषि अनुसंधान परिषद- भारतीय चरागाह और चारा अनुसंधान संस्थान, झाँसी ने बाजरा नेपियर हाइब्रिड का उत्पादन करने वाला दुनिया का पहला उपजाऊ बीज विकसित किया है। "बाजरा-नेपियर हाइब्रिड में बायोमास से संबंधित लक्षणों के फ्रेमवर्क लिंकेज मानचित्र और आनुवंशिक विच्छेदन का निर्माण" शीर्षक वाला वर्तमान अध्ययन केंद्रीय अनुसंधान फार्म, फसल सुधार प्रभाग, भारतीय कृषि अनुसंधान परिषद- भारतीय चरागाह और चारा अनुसंधान संस्थान, झाँसी में खरीफ 2022 के दौरान किया गया। वर्तमान जांच में, लिंकेज मानचित्र के निर्माण के लिए कुल 250 इमोर्टलैसेड F₂ पापुलेशन को संवर्धित रैंडमाइज्ड ब्लॉक डिजाइन (RCBD) में प्रत्यारोपित किया गया और 22 बायोमास से संबंधित लक्षणों के लिए फेनोटाइपिक लक्षण वर्णन किया गया। जनसंख्या में भिन्नता को दर्शाने के लिए आनुवंशिक परिवर्तनशीलता, आनुवंशिकता और आनुवंशिक उन्नति, सहसंबंध अध्ययन, प्रमुख घटक विश्लेषण और क्लस्टर विश्लेषण किए गए। बहुरूपता के लिए पैतृक की स्क्रीनिंग के लिए कुल 217 SSR मार्करों का उपयोग किया गया। इनमें से 138 ने पैतृक बहुरूपता दिखाई और यादृच्छिक रूप से चयनित जीनोटाइप की मदद से जनसंख्या में सत्यापन की जाँच की। 138 में से 100 को अंततः जीनोटाइप किया गया। बाजरा नेपियर हाइब्रिड का पहला लिंकेज मानचित्र 88 SSR मार्कर के साथ बनाया गया। इसका विस्तार 858.08 cM और औसत मार्कर घनत्व 9.75 cM है। SSR मार्करों को सात लिंकेज समूहों के बीच असमान रूप से वितरित किया गया। विचरण के विश्लेषण से सभी 22 मापे गए लक्षणों के लिए महत्वपूर्ण अंतर पता चला। पत्ती की लंबाई, पत्ती की चौड़ाई और स्पाइक की लंबाई लक्षणों पर भी पर्यावरण का महत्वपूर्ण प्रभाव देखा गया। कम्पोजिट इंटरवल मैपिंग (CIM) द्वारा बायोमास से संबंधित लक्षण के लिए QTLs की पहचान करने के लिए इमोर्टलैसेड F₂ पापुलेशन से फेनोटाइपिक डेटा का उपयोग किया गया। 13 बायोमास से संबंधित लक्षणों में कुल 33 QTL (LOD $\geq$ 3.0) का पता लगाया गया। QTLs की फेनोटाइपिक भिन्नता (PVE) 10.8 से 70.64 प्रतिशत तक थी। स्पाइक लंबाई के लिए QTL द्वारा उच्चतम फेनोटाइपिक भिन्नता 70.64% की व्याख्या की गई।

निष्कर्ष में, यह परिकल्पना की गई है कि बायोमास से संबंधित लक्षणों के लिए 88 एसएसआर मार्करों और 33 QTLs के साथ सुदृढ़ वर्तमान लिंकेज मैप इस फसल प्रजातियों की आगे आनुवंशिक वृद्धि के लिए प्रजनकों को जीनोमिक्स उपकरण प्रदान करेगा। इस प्रकार, वर्तमान अध्ययन बीएन हाइब्रिड प्रजनन कार्यक्रमों में उपयोग के लिए QTL के आगे सत्यापन और ठीक मानचित्रण के लिए एक मजबूत आधार के रूप में काम करेगा।


ए आर एस एस एच गुप्ता
विद्यार्थी


डॉ. मनीत राणा
प्रमुख सलाहकार

BRIEF BIODATA OF STUDENT

Name : Alapati Revanth Satya Sai Hanuman Gupta
Father's Name : Sri.Venkateswarlu
Mother's Name : Smt. Lakshmi Surekha
Date of Birth : 23rd December, 1998
Permanent Address : D.No: 4-30, Alluru, Pittalavani Palem Mandal, Bapatla District, Andhra Pradesh, INDIA, 52314.
Languages known : English, Telugu (naïve), Hindi

Academic Qualifications:

Examination Passed	Month, Year	Board/University	Marks (%)	Division
High school	May., 2014	Sri Tagore's High School	95	1 st
Intermediate (10+2) (Bi.P.C)	Mar., 2016	Sri Chaitanya Junior College	97.5	1 st
B. Sc. (Hons) Agriculture	June., 2021	ANGRAU, Rajamahendravaram	88.7	1 st
M. Sc. (Ag.) Genetics and Plant Breeding	Aug., 2023	RLBCAU, Jhansi	86.2	1 st

Thesis Title in M.Sc. – Construction of framework linkage map and genetic dissection of biomass related traits in Bajra-Napier Hybrid

Skills:

Research : DNA Isolation, PCR, Gel Electrophoresis, Genotyping, Field phenotyping
 Computer : M.S Word, MS Excel, Power Point presentation, Adobe Acrobat, QTL cartographer, Map chart, Join map, Image J, StatistiXL, R programming.
 Languages : English, Telugu (Native) and Hindi

Affiliations & Awards

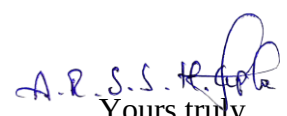
National Talent Scholarship (Indian Council of Agricultural Research) 2021-2023
 Best Student Award (Acharya N G Ranga Agricultural University) 2017-2021

Publications

2 Book Chapters, 3 Popular articles

DECLARATION

I do hereby declare that the information given above is true to the best of my knowledge and belief.


 Yours truly
 A R S S H Gupta