RESEARCH ARTICLE

Genetic analysis for green fodder quality characters in concurrence of heterosis and genotype-by-trait biplot in bread wheat (*Triticum aestivum* L.)

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Abstract

Animal health is of utmost importance, whereas green fodder supply is limiting day-by-day. In the present investigation, eight diverse wheat varieties were crossed in half-diallel fashion and were analysed along with 28 F1s for green fodder digestibility and crude protein in the Bundelkhand region of Madhya Pradesh and Uttar Pradesh. The parental genotype NIAW34 showed the highest organic matter (91.65%), followed by PBW723 (91.47%) and GW322 (91.30%). Acid detergent fiber (ADF), cellulose and lignin contents ranged from 26.86-33.89%, 2.59–28.02% and 2.91–4.40%, respectively for parents and hybrids. Hemicellulose content varied from 27.60–36.58% and the parents namely, HI1544 (30.52%) and GW322 (29.91%) were found promising with the hybrids viz., HD3086/DBW110, HI1544/ NIAW34 and GW322/NW5054. The study revealed that wheat green fodder quality was comparable with the sorghum and maize crops. Organic matter and neutral detergent fiber revealed a preponderance of additive gene action, whereas protein and nitrogen contents, cellulose, lignin, acid detergent fiber and hemicellulose showed an average degree of dominance more than unity. The narrow leaf wheat genotype DBW110 exhibited higher cellulose and lignin contents, providing insight for on-field selection. The *per s*e performance and genotype by trait biplot revealed the potential of wheat variety, GW322 for multiple fodder digestibility traits.

Keywords: Wheat, Neutral detergent fiber, Acid detergent fiber, Biplot, Heterosis.

Introduction

Wheat (*Tritium aestivum* L*.)* is the world's basic staple food crop, which provides nearly 20% of the total human intake calories (Tomar et al. 2014; Wang et al. 2021). During 2021, globally, wheat ranked third after maize (1210.23 m t) and rice (787.29 m t) in terms of production with 770.87 m t grains from 220.75 m ha area (FAOSTAT 2023). India is the second largest wheat producer in the world and recorded an all-time high wheat production of 112.74 m t during the year 2022-23 (Anonymous 2023). Wheat is a multiple purpose crop as its grains are typically milled into flour, which is then used to make various products, a good source of fiber, industrial products, as feed and forage (dry *bhusa*). Agriculture and animal husbandry are inter-related and essential elements of rural livelihood (Dagar et al. 2017; Singh et al. 2022). These directly impact the rural life, urban health and food security of nearly a billion people worldwide by addressing mineral availability for combating malnutrition (Hurst et al. 2007; Downing et al. 2017). Since ancient times, livestock has been a significant factor of human civilizations and India has the largest livestock population in the world. In rural areas, around 70% of households depend on livestock income and further on agriculture sector for their livelihood. The deficiency of dry fodder (10.95%), green fodder (35.6%) and concentrate feed (44%) has already been emphasized in India (IGFRI Vision 2050). The projected demand for green and dry feed has been estimated of 1012 and 631 million

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tonnes by the year 2050, respectively. Therefore, the green fodder supply must increase by 1.69%/annum, where the cultivable fodder area accounts for only 4% of the total cultivated land in the country (Dagar et al. 2017; Meena et al. 2018). Small land holding and resource availability are other issues leading to underproductive and weak livestock in semi-arid and tropics. Marginal farmers use crop residues for livestock feeding, which are reported to be low in nutritional quality. Further, the farmers are intended to grow crops having high productivity with market driven demand for easy sale-out of the products.

Wheat is a staple food crop occupying a large area in the country and has ensured procurement from government or private agencies. Besides nutritional and industrial importance, it can also be a viable and economical option for green fodder in hilly and plain areas. Along with straw quality wheat green fodder has an important role in livestock feeding as it is nutritious for the livestock feed. The green fodder availability can be increased after taking a cut at 55 to 60 days after sowing as the first cutting (Sharma et al. 2019). The period March-May is the peak period for the availability of wheat straw, the period just before the harvest time, in December to February, green fodder plays an important role in meeting out the scarcity of fodder when the cost and availability of straw are exorbitantly high (Teufel et al.2010). The crude protein and forage digestibility are the main quality-deciding parameters, including forage intake capacity (Murray et al. 2008; Li et al. 2018). Forage digestibility is chiefly determined by the cellulose, acid detergent fiber (ADF), neutral detergent fiber (NDF), hemicellulose and lignin contents (Li et al. 2018; Wang et al. 2016). It has been reported earlier (Firdous and Gilani 2001; Chaudhary et al. 2016; Chakravarthi et al. 2017) that wheat green fodder is equally comparable with the other forage crops, like sorghum and maize for green fodder digestibility. However, the information on the genetics of wheat green fodder quality traits is scanty. The present investigation was, therefore, carried out to investigate *elite* wheat varieties and their developed crosses for green fodder quality traits.

Materials and methods

Experimental materials site and experimental design

Eight *elite* diverse bread wheat varieties namely, HD3086 (P1), HI544 (P2), DBW110 (P3), GW322 (P4), K1006 (P5), NW5054 (P6), PBW723 (P7) and NIAW34 (P8) were considered for the study (Table 1). The varieties HD3086, HI1544, GW322, K1006 and NW5054 are high foliage varieties and are under cultivation in the Bundelkhand region of Madhya Pradesh and Uttar Pradesh. The PBW723 is an improved version of ruling wheat variety PBW343 and was considered as reported by Sharma et al. 2019, whereas NIAW34 has a drought tolerance, much needed in Bundelkhand region. These eight genotypes were crossed during *rabi*, 2019-20 in half diallel fashion (excluding reciprocals) and planted at the Research Farm of Rani Lakshmi Bai Central Agricultural University, Jhansi, Uttar Pradesh, India during *rabi,* 2020-21. Jhansi, is geographically located between 25º31'02.5''N latitude to 78º33'05.11'' E longitude and at an elevation of 227 metres above mean sea level. The 28 $F₁$ hybrids and 8 parents (Table 2) were examined in randomized complete block design with 2 replications in paired rows of 2 m length and 30 cm row to row distance..

Green fodder quality analysis

For green fodder nutritional quality analysis, leaves of two to three plants were cut at 55 days after sowing and dried in the oven, then powdered using a mortar and pestle for further investigation. Organic matter (OM) and crude protein (CP) were calculated using the (AOAC 2005) method. The difference between the oven-dried sample weight and the ash weight was used to compute the amount of organic matter in the plant. The Kjeldahl method was used to estimate crude protein. To obtain nitrogen content (N) and crude protein (CP) was divided by 5.70 and the resultant value was nitrogen content. Acid detergent fiber (ADF), neutral detergent fiber (NDF), cellulose (CL) and lignin (L) were estimated using the (Van Soest et al. 1991) method.

GC	Parent/cross	GC	Cross	GC	Cross	GC	Cross
G1	HD 3086	G10	HD 3086/DBW 110	G19	HI 1544/ NW 5054	G ₂₈	GW 322/ NW 5054
G ₂	HI 1544	G11	HD 3086/ GW 322	G20	HI 1544/ PBW 723	G29	GW 322/ PBW 723
G ₃	DBW 110	G12	HD 3086/K 1006	G ₂₁	HI 1544/ NIAW 34	G30	GW 322 /NIAW 34
G4	GW 322	G13	HD 3086/NW 5054	G22	DBW 110/ GW 322	G31	K1006/NW 5054
G5	K1006	G14	HD 3086/ PBW 723	G23	DBW 110/K 1006	G32	K 1006 / PBW 723
G6	NW 5054	G15	HD 3086/ NIAW 34	G ₂₄	DBW 110/NW 5054	G33	K 1006 / NIAW 34
G7	PBW 723	G16	HI 1544/ DBW 110	G ₂₅	DBW 110/ PBW 723	G34	NW 5054/ PBW 723
G8	NIAW 34	G17	HI 1544/ GW 322	G ₂₆	DBW 110/ NIAW 34	G35	NW 5054 /NIAW 34
G9	HD 3086/HI 1544	G18	HI 1544/K 1006	G27	GW 322 /K 1006	G36	PBW 723 /NIAW 34

Table 2. Details of genotypic code and crosses for biplots

GC=Genotype code

After the ADF estimation, the sample left in the crucible was treated with 72% H₂SO₄ and then a glass rod was taken to swirl the solution and the solution was kept for 3 hours and then washed three times with boiled water to eliminate the acid. The crucibles were cleaned and dried in a hot air oven for 24 hours before weighing. After H_2 SO₄ treatment, the cellulose sample was dried in an oven and then burned in a muffle furnace and the ash was quantified. This lignin was made from acid detergent fibre. This approach employs detergents that combine with protein to render it soluble and a chelating agent to remove heavy metal and alkali contaminants. Hemicellulose (HCL) was expressed as a percentage by subtracting the ADF values from the NDF estimates.

Statistical analysis

The analysis of variance, combining ability analysis using Griffing (1956) method 2 (excluding reciprocals) and Model-I (Fixed effect model) and heterosis were computed following Singh and Chaudhary (1977). The correlation coefficients were computed following standard procedures. The genotype by trait and comparison biplots were generated using R version 4.1.3 following column metric preserving SVP, tester-centered genotype and genotype-environment interaction (G+GE) without any scaling. The scatter plot and heat map were extracted using R Studio version 2023.

Results

Per se performance for green fodder quality traits

The analysis of variance showed significant mean squares for all the characters (P<0.01) and further partitioning of mean squares revealed adequate genetic variation among the treatments. Average organic matter for parents and F₁s was observed as 91.11%, which ranged from 89.77 to 93.62% (Table 3). Among the parents, the genotype NIAW34 showed the highest organic matter (91.65%), followed by PBW723 (91.47%) and GW322 (91.30%), whereas the cross combinations HI1544/GW322 (93.62%), DBW110/GW322 (92.47%) and HD3086/PBW723 (92.26%) were found promising for organic matter. The parental genotypes HI1544 and GW322 revealed high crude protein and nitrogen contents of 15.43 and 2.71% each, followed by PBW723 (15.28 and 2.68%) and NIAW34 (15.23 and 2.67%). ADF, cellulose and lignin contents showed mean values of 30.0, 24.64 and 3.59%, which ranged from 26.86 to 33.89%, 2.59 to 28.02% and 2.91 to 4.40%, respectively for parents and hybrids (Fig. 1). The parents GW322 (30.20%) and HI1544 (31.69%), and the

Table 3. Parental and hybrid per se performances for green fodder quality traits

Characters		Parents		$F_{1}S$	
	GM	Mean \pm SE(m)	Range	Mean \pm SE(m)	Range
Organic matter (%)	91.11	90.88 ± 0.47	$89.90 - 91.65$	91.17 ± 0.47	$89.77 - 93.62$
Crude protein (%)	15.52	15.12 ± 0.58	$14.60 - 15.43$	15.63 ± 0.58	$13.06 - 17.95$
Nitrogen content (%)	2.72	2.65 ± 0.10	$2.56 - 2.71$	2.74 ± 0.10	$2.29 - 3.15$
Acid detergent fiber (%)	30.0	32.41 ± 0.70	$30.20 - 33.89$	29.31 ± 0.70	$26.86 - 32.15$
Cellulose (%)	24.64	26.71 ± 0.67	$24.76 - 28.02$	24.05 ± 0.67	$21.59 - 26.99$
Lignin (%)	3.59	3.92 ± 0.20	$3.67 - 4.14$	3.49 ± 0.20	$2.91 - 4.40$
Neutral detergent fiber (%)	60.67	61.42 ± 1.14	$60.11 - 62.24$	60.46 ± 1.14	$56.80 - 65.47$
Hemicellulose (%)	30.68	29.01 ± 1.12	$27.60 - 30.53$	31.15 ± 1.12	$27.93 - 36.58$

Fig. 1. Scatter plot and correlation matrix of the variables

crosses HD3086/HI1544 (27.41%), HI1544/GW322 (27.95%) and HD3086/DBW110 and HD3086/GW322 (27.97% each) were favourable for low ADF content. The parents GW322 and HI1544 showed better *per se* for cellulose, whereas PBW723 and GW322 were found better for lignin content. The NDF content showed mean value of 60.67% (Table 3) and the crosses K1006/NIAW34 and DBW110/GW322 exhibited lower NDF. Hemicellulose content ranged from 27.60 to 36.58% and the parents, namely, HI1544 (30.52%) and GW322 (29.91%) were found promising with the hybrids *viz*., HD3086/DBW110, HI1544/NIAW34, GW322/NW5054 and HD3086/HI1544. Among the parents, the genotype GW322 showed better *per se* and favorable allele additivity for nearly all the green forage quality traits, whereas the parental genotypes PBW723 and NIAW34 revealed favorable gene constellations for organic matter, crude protein and nitrogen contents. The heat map developed from the mean data was useful in the visualization of trait effects and depicted the highest values average values for organic matter (OM) followed by NDF and HCL (Fig. 2).

Heterosis and heterobeltiosis

For organic matter the mean mid-parent heterosis was observed as 0.32% and the positive mean heterosis was recorded for 17 crosses. However, it was positively significant in only two crosses, namely, HI1544/GW322 (2.73**) and DBW110/GW322 (2.07*). The heterobeltiosis was positive

Fig. 2. Heat map of the green fodder quality traits in wheat

in 13 crosses and the hybrids *viz*., HI1544/GW322 (2.54**) and HD3086/DBW110 (1.45*) showed positive significant heterosis over the better parent. The crosses GW322/ PBW723, DBW110/GW322, DBW110/NIAW34, PBW723/ NIAW34 and HI1544/NIAW34 and HD3086/K1006 showed the maximum mid-parent and better parent heterosis for crude protein and nitrogen contents. The means of average heterosis and heterobeltiosis for ADF were recorded as -9.54 and -11.42%, which ranged from -16.32 to -1.27 and -20.74 to -3.84%, respectively (Table 4). Based on parent and better parent heterosis the cross, HI1544/NW5054 was not found with positive gene nicking ability for ADF. In the present study, the mean mid-parent heterosis values for cellulose, lignin and NDF ranged from -18.19 to 0.86, -25.72 to 9.52 and -7.60 to 5.75%, respectively. The hybrids, DBW110/GW322 (-18.19**), followed by DBW110/K1006 (-17.33**), HD3086/ DBW110 (-15.05**), DBW110/NIAW34 (-14.60**) and K1006/ NIAW34 (-14.02**) were top five performers based on mid parent heterosis for cellulose content. For lignin content the cross combinations, HD3086/DBW110, HD3086/HI1544, NW5054/PBW723, K1006/PBW723 and HD3086/NW5054 were found promising with high heterobeltiosis. For NDF content, mean mid-parent heterosis and better parent heterosis were exhibited as -1.56 and -2.40%, where the crosses, HI1544/PBW723, DBW110/NIAW34, K1006/NIAW34 and DBW110/GW322 revealed high average and better parent heterosis. The mid and better parent heterosis for hemicellulose content ranged from -7.42 to 30.78 and -8.50 to 30.20%, respectively. The cross, HD3086/DBW110 (30.20**), followed by HI1544/NIAW34 (18.35**), K1006/ PBW723 (14.51**) were recorded with high average and better parent heterosis.

Gene effects analysis

The analysis of variance depicted significant GCA and SCA effects for all the traits except, GCA effects for NDF. SCA effects for crude protein, nitrogen content, ADF, lignin, NDF and hemicellulose were estimated to be higher than the GCA effects, indicating the prevalence of the non-additive gene effects. For organic matter, the parental line GW322 showed significant and positive GCA effects (0.53**) and the parents, NIAW34 and HI1544 exhibited non-significant and positive GCA effects. The genotypes GW322 and NIAW34 were found promising with significant and positive GCA effects for crude protein and nitrogen contents (Table 5). The parental genotypes GW322 and HD3086 depicted promising performance for ADF, whereas for cellulose content the genotype GW322 was identified as good general combiner. The variety HD3086 followed by PBW723 were found promising for lignin content and the genotypes HD3086 and HI1544 could be regarded as better parents for hemicellulose content. The crosses, GW322/PBW723, HD3086/K1006, NW5054/PBW723, HI1544/NIAW34, DBW110/

Table 4. Per cent mean and range of average heterotic and heterobeltiotic combinations

GW322, DBW110/K1006, DBW110/NIAW34 and PBW723/ NIAW34 revealed better SCA effects for crude protein and nitrogen content. For ADF and cellulose contents, the hybrid, K1006/NIAW34 (-2.33** and -1.94**), followed by DBW110/K1006 (-2.26** and -1.91**), DBW110/GW322 (-2.19** and -1.86**) and HD3086/HI1544 (-2.14** and -1.69**) exhibited desirable SCA effects. For hemicellulose content, the crosses, HD3086/DBW110 (5.77**), followed by K1006/ PBW723 (4.57**), HI1544/NIAW34 (4.43**), K1006/NW5054 (2.99**) and GW322/NIAW34 (2.34**) were found with preponderance of the SCA effects. Based on GCA effects, the parental genotype GW322 could be regarded superior for organic matter, crude protein, nitrogen content, ADF and cellulose content. The hybrids HD3086/K1006 and DBW110/ GW322 showed desirable SCA effects (Table 5).

Biplot analysis

The initial two components of genotype by trait (GT) and comparison biplots captured 89.35 % of the total variation. The GT showed narrow variation among parents and crosses

Fig. 3. Genotype by trait GGE Biplot for fodder quality traits

Fig. 4. Comparison of HD3086 (G1) and GW322 (G4) by GGE Biplot

for lignin content (L), organic matter (OM), crude protein (CP) and nitrogen content (N) (Fig. 3). The digestibility parameters were grouped in separate quadrant. The biplot generated for comparison of leading variety HD3086 (G1) with the promising genotype GW322 (G4) confirmed alignment of GW322 towards the centre (Fig. 4). The characters HCL and NDF were found with greater variability, while the traits *viz*., lignin, crude protein, organic matter and nitrogen content showed narrow variation.

**Significant at 5 percent level*

***Significant at 1 percent level*

Correlations

The scatter plot presented in Fig. 1 depicted range of the studied variables and correlations. Organic matter showed negative and significant correlations with ADF (-0.33*) and cellulose content (-0.39*). The crude protein and nitrogen content were found to be correlated positively, whereas crude protein exhibited negative and non-significant correlations with ADF, cellulose and lignin content. ADF showed the highest positive correlations with cellulose content (0.98*), followed by lignin (0.51*) and NDF (0.44*), while it showed a negative correlation with hemicellulose content (-0.34*). The cellulose content was positively associated with NDF (0.46**) and hemicellulose showed negative correlations with cellulose content.

Discussion

The Bundelkhand region of Madhya Pradesh and Uttar Pradesh in India is known for low water availability, erratic rainfall and terminal heat. The livestock sector contributes significantly in rural livelihood with crop ecology. Therefore, animal health is of much importance and can only be ensured by quality dietary intake. The farmers are marginal with very small land holdings and cannot afford cultivation of separate high water requiring *rabi* fodder crops and wheat can serve a potential dual-purpose crop in the region. The wheat varieties under cultivation have high foliage coupled with good canopy coverage and can be explored for green fodder. In the present study, organic matter ranged from 89.90 to 91.65% and it was hypothesized that the genotype DBW110 having comparatively narrow leaves at the tillering and late jointing stage had low organic matter. The genotypes GW322 and HI1544 and crosses, namely, HD3086/ HI1544, HI1544/GW322, HD3086/DBW110 and HD3086/ GW322 were observed with low ADF content and can be suggested for consideration with higher digestibility. Yang et al. (2021) reported that fiber content (ADF and NDF) and crude protein are major indicators of forage quality affecting nutrition intake and digestibility for animal feeding (Ball et al. 2001; Wang et al. 2016). Low NDF is required for good feed intake, whereas ADF and ADL are negatively associated with fodder digestibility. The genotypes GW322, HD3086 and K1006, can positively impact high animal feed intake as these depicted low NDF values. The ADF and NDF increase with plant ageing and it is also evident in wheat as Joshi et al. (2019) reported ADF and NDF values of 50.9 and 75.6%, respectively in wheat. The ADF and NDF values increased by 70 and 25% with plant ageing. Hence, wheat green fodder is better for digestibility and intake, improving animal health. Besides, the wheat green fodder is also comparable with the other forage crops like sorghum and maize as the ADF, NDF, cellulose and hemicellulose values obtained were relatively better as reported by Firdous and Gilani, 200; Chaudhary et al. 2016; Chakravarthi et al. 2017. Sharma et al. (2019) reported that green fodder cutting especially in tall wheat varieties is economical for taking green fodder and as well grain yield with-out any yield reduction.

Accumulation of favourable complementary alleles in the hybrids coming from dispersive parents leads to the level of heterosis in crop plants. Here, the hybrids, GW322/ PBW723, DBW110/NIAW34, DBW110/GW322 and NW5054/ NIAW34 showed heterotic effects for crude protein, nitrogen per cent and hemicellulose content. These hybrids involved combination of all the eight parents, however only the parents GW322, HI1544, PBW723 and NIAW34 were promising for crude protein, nitrogen per cent and hemicellulose content. Similarly, the hybrids, HD3086/ K1006, HD3086/NW5054, HI1544/GW322, HI1544/K1006, HI1544/PBW723 and DBW110/GW322 were heterotic for ADF, ADL, cellulose and NDF. This revealed that the parents of Low × Low, Low × Medium *per se* were also having gene dispersion and further complementarity of the genes in the hybrids for the above traits. Such parents would be more useful in generating diverse heterotic combinations in mating with other wheat varieties and further delineating underlying QTLs at molecular level. Joshi et al. (2019) mapped QTLs for ADF and ADL on the chromosome 2B, while some marker traits associations were detected on the wheat chromosomes, 1A, 2B, 3A, 5A and 5B explaining low to medium phenotypic variations. Esposito et al. (2022) reported minor genes on chromosomes 5A, 2B and 2A for ADF, ADL and cellulose, respectively for straw quality in wheat. The biplots generated also corroborated the obtained correlations of fodder intake and digestibility traits with CP and OM. The GT biplots were useful in identifying trait associations and genotypic discrimination (Yan et al. 2000; Yan et al. 2007; Kumar et al. 2016)

Estimation of GCA and SCA helps in determining desirable genotypes, where GCA demonstrates the ability of different genotypes to dependably inherit their genetic potential to $\mathsf{F}_{\!{}_1}$ progeny when used as parents. The later reveals unexpected favorable or unfavorable genic interactions in different genotypes. In the present investigation, the SCA effects were prevalent for most of the traits, except organic matter and NDF. The degree of dominance was higher for the traits with incremental SCA effects and indicated complex inheritance for green fodder quality traits in wheat. The parent GW322 was promising for organic matter, protein and nitrogen content, ADF and cellulose content. However, with one cross combination of GW322 only one cross, DBW110/GW322 could find place among the top five performing hybrids. It indicated the non-correspondence of GCA *vs*. SCA effects for fodder quality traits in wheat. Among the top five performing hybrids, only one hybrid was of high \times high combination and rest four crosses were of $L \times H$ origin. Somegowda et al. (2021) also reported a non-significant association of the

agronomic and fodder quality traits in sorghum and the utilization of contrasting genotypes ($L \times H$) could be useful in identifying important genes controlling the fodder quality traits. Green fodder availability is scarce in plains with low irrigation facilities and in mid and higher hills during winter, where irrigation demanding fodder crops are not feasible. The subsistence farmers with small holdings are largely unable for fodder production and the wheat can serve an additional advantage. However, all the genotypes cannot be directly utilized for green fodder production and the present investigation will be highly useful for delineating gene effects for further genetic and molecular analyses. Kaur et al. (2017) studied cellulose content in wheat and identified firmly associated SNP markers linked to the genes *viz*., *Auxininduced protein 5NG4* and *β-tubulin* for cellulose content in wheat. Correlation between ADF with OM, CP₁N and HCL were negative here, while ADF found positively associated with Ch, L and NDF. These correlations are now for green fodder quality in wheat have not been reported earlier.

In conclusion, the organic matter and NDF revealed a preponderance of additive gene action, whereas protein and nitrogen contents, cellulose, lignin, ADF and hemicellulose were prevalent with dominance gene action and indicated scope of regenerative and heterotic crosses in wheat. It could be hypothesized that the narrowed-leaf wheat genotypes had high cellulose and lignin contents with low organic matter and can provide field selection criteria for breeders. In view of the scanty information on wheat green fodder quality genetics, the present investigation will be highly useful for future breeding programmes.

Authors' contributions

Conceptualization of research (VK, MKS); Designing of the experiments (VK, MKS, SK); Contribution of experimental materials (VK, SS, KKS); Execution of field/lab experiments and data collection (MKS, SS, KKS); Analysis of data and interpretation (VK, PS, SK, SS, MKS); Preparation of the manuscript (VK, MKS).

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