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Genetic aspects of nitrogen metabolism in barley (*Hordeum vulgare* L.)

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Abstract
Barley (*Hordeum vulgare* L.) is gaining a place among cereals as a food and feed for human and animal respectively in recent time because of its nutritive and malting properties. It is known that the crop can be grown efficiently even under harsh environment due to genetic makeup. Nitrogen an essential nutrient influences the growth and yield of barley. Among the constraints under this condition are water and nutrient, especially nitrogen availability. Therefore, an attempt has been made to understand the genetics of nitrogen metabolism vis-à-vis nitrogen utilization efficiency (NUE), nitrogen transportation, nitrogen assimilation, remobilization in different parts of plant as well as in grains. Thus for improvement of barley genotypes for low water, low nitrogen and high salinity i.e. harsh environment, a detailed analysis of genetic components are being undertaken for tolerant genotypes. Higher expression of the transporter HvNRT2/3 genes, especially *HvNRT2.1*, *HvNRT2.5*, and *HvNRT3.3* also the expression levels of N assimilation genes including *HvNIA1*, *HvNIR1*, *HvGS1_1*, *HvGS1_3*, and *HvGLU2* increased significantly nitrogen use efficiency(NUE) in barley. Partial sequences of five genes related to N-metabolism in barley (*Hordeum vulgare* L.) were obtained, i.e. nitrate reductase 1, glutamine synthetase 2, ferredoxin-dependent glutamate synthase, aspartate aminotransferase and asparaginase. Two to five haplotypes in each gene were discovered in a set of 190 varieties. Findings of 33 SNP markers allowed the genotyping of all these barley varieties consisting of spring and winter types. From correlation analyses it is confirmed that GOGAT was related to G6PDH; GDH and APX with PEPC in"100/1B" under moderate salinity; severe salinity is better correlated to GDH with G6PDH and PEPC.

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Keywords: barley (*Hordeum vulgare* L.), nitrogen use efficiency (NUE), transporters, nitrogen metabolic enzymes/genes, low nitrogen tolerance, drought, salinity

Introduction

Barley is a staple crop known for its great adaptability to harsh environments. It was one of the first domesticated crops and is the fourth most productive cereal crop after rice, wheat, and maize (FAOSTAT). Barley (*Hordeum vulgare* L.) shows a very large genetic diversity and is grown under a large array of environmental and soil conditions with areas of high altitudes and latitudes as well as in desert regions (Ryan and Sommer, 2012; Muñoz-Amatriaín *et al*., 2014; Dawson *et al*., 2015) [39, 30, 10]. The global production of barley amounted to about 151.62 million metric tons in the 2022/2023 crop year, increasing from 145.37 million metric tons in 2021/2022.

United States	3,795,650	
	United Kingdom	7,385,000
	Ukraine	5,608,170
	Turkey	8,500,000
	Sweden	1,509,500
	Spain	7,029,720
	Russia	23,393,510
	Romania	1,706,650
	Poland	2,782,010
	Kazakhstan	3,287,240
	Italy	1,158,410
	Ireland	1,549,860
	Iran	3,000,000
	India	1,371,360
	Hungary	1,590,740
	Germany	11,207,100
	France	11,285,440
	Finland	1,467,600
	Ethiopia	2,400,000
	Denmark	4,122,600
	Czechia	1,877,360
	China	1,960,000
	Canada	9,986,681
	Belarus	1,100,000
	Azerbaijan	1,069,446
	Australia	14,377,284
	Argentina	5,279,608
	Algeria	1,600,000

Barley is used for animal feed, human consumption, and malting. It is gaining value as a nutritious food, not only for its original flavor but especially because of its high content in β-glucans and low gluten (Baik and Ullrich, 2008; Chutimanitsakun *et al*., 2013) [4, 9]. Barley is considered for several benefits to human health, such as reduction of blood cholesterol and glucose levels as well as weight loss by increased satiety, control of heart disease, and type-2 diabetes (Baik and Ullrich, 2008) [4]. In some parts of the world, such as Ethiopia, North Africa, and Asia, it is used as human food more frequently than in the rest of the world (Baik and Ullrich, 2008) [4].

Mediterranean climate and soils impose drastic constraints on agriculture. Barley is one of the best-adapted species to the Mediterranean conditions (Pswarayi *et al*., 2008) [35]. Climate change and the growing Mediterranean population will further increase on barley culture in a near future (Cammarano *et al*., 2019) [8]. Fortunately, barley shows great potential for biomass production under Mediterranean climates. As is the case for most cereals, barley yields are strongly dependent on nitrogen fertilization (Oscarsson *et al*., 1998; Sedlář *et al*., 2011; Stupar *et al*., 2017) [33, 40, 45]. Importantly, it is noted that nitrogen fertilization impacts plant tolerance to abiotic and biotic stresses (Fagard *et al*., 2014; Abid *et al*., 2016; Mur *et al*., 2017; Ding *et al*., 2018; Verly *et al*., 2020) [14, 1, 31, 12, 48]. The genetic diversity in terms of barley tolerance to nitrogen starvation has been explored (Oscarsson *et al*., 1998; Górny, 2001; Sinebo *et al*., 2004; Quan *et al*., 2016, 2019; Karunarathne *et al*., 2020) [33, 18, 43, 36, 37, 23, 24]. However, few data are available concerning the diversity of molecular responses of barley to nitrogen limitation (Møller *et al*., 2011; Quan *et al*., 2016, 2019; Karunarathne *et al*. 2020, 2021)(Fig.1) [36, 37, 23, 24].

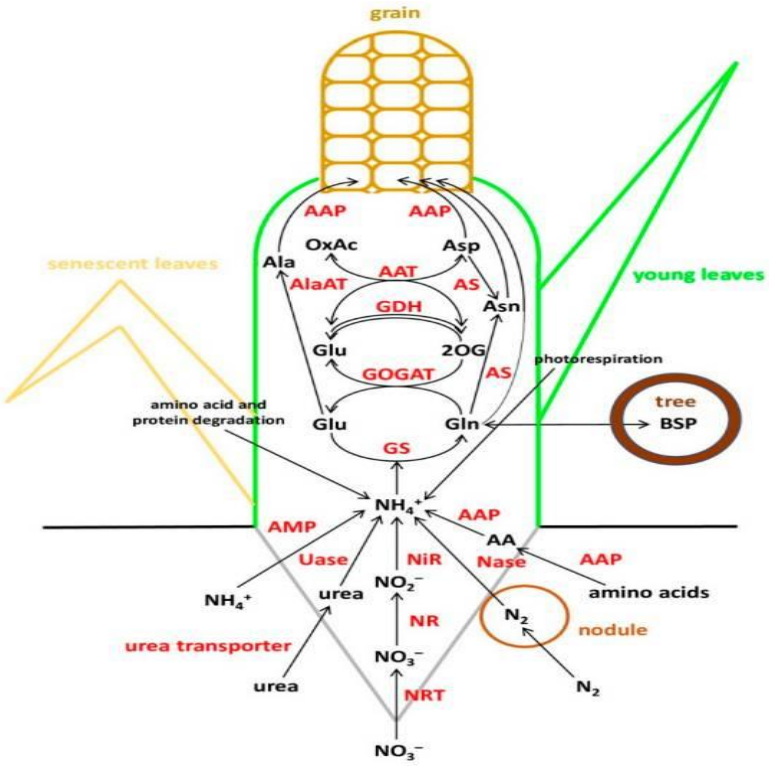

Fig 1: Nitrogen metabolism in plant

Nitrogen use efficiency (NUE)

Over application of nitrogen (N) fertilizers to crops ultimately causes N pollution in the ecosphere. Studying the response of plant growth and N uptake to low-N stress may aid in elucidating the mechanism of low N tolerance in plants and developing crop cultivars with high nitrogen use

efficiency (NUE). A high-NUE mutant line A9-29 and the wild-type barley cultivar Hua30 were subjected to hydroponic culture with high and low N supply, and the dry weight, N accumulation, root morphology, and expression levels of the potential genes involved in nitrate uptake and assimilation were measured at seedling stage. The results showed that under low-N conditions, A9-29 had a higher dry weight, N content, N influx rate and larger root uptake area than Hua30. Under long-term low-N stress, compared with Hua30, A9-29 demonstrated higher expression of the HvNRT2/3 genes, especially *HvNRT2.1*, *HvNRT2.5*, and *HvNRT3.3*. Similarly, the expression levels of N assimilation genes including *HvNIA1*, *HvNIR1*, *HvGS1_1*, *HvGS1_3*, and *HvGLU2* increased significantly in A9-29. It was suggested that the larger root area and the upregulation of nitrate transporter and assimilation genes may contribute to greater N uptake capacity for plant growth and N accumulation in responding to long-term low-N stress (Table 1and Fig.2). These findings may aid in understanding the mechanism of low N tolerance and developing barley cultivars with high-NUE (Gao *et al*., 2021) [16].

Table 1: Effect of HN and LN supply on root morphology between Hua30 and A9-29 on the 7th day of treatment. Mean ± SD (n = 5) with the same line followed the different letters (after GAO, *et al*., 2021) [16].

Root traits	Treatment	A9-29	Hua30
Root length (cm)	HN	238.24 ± 20.93a	170.44 ± 10.38b
	LN	360.70 ± 12.65a	240.17 ± 13.31b
Main root length (cm)	HN	20.25 ± 1.12a	14.69 ± 0.52b
	LN	29.43 ± 0.87a	18.30 ± 1.51b
Root surface area(cm^2)			
	HN	16.42 ± 1.02a	11.98 ± 0.48b
	LN	25.30 ± 1.52a	16.01 ± 1.01b
Root volume (cm^3)	HN	0.14 ± 0.01a	0.11 ± 0.01b
	LN	0.24 ± 0.04a	0.19 ± 0.04a
Root average diameter (cm)			
	HN	0.27 ± 0.01b	0.33 ± 0.01a
	LN	0.33 ± 0.04b	0.49 ± 0.05a
Root number	HN	7.4 ± 0.24a	7.8 ± 0.2a
	LN	7.6 ± 0.24a	7.8 ± 0.37a

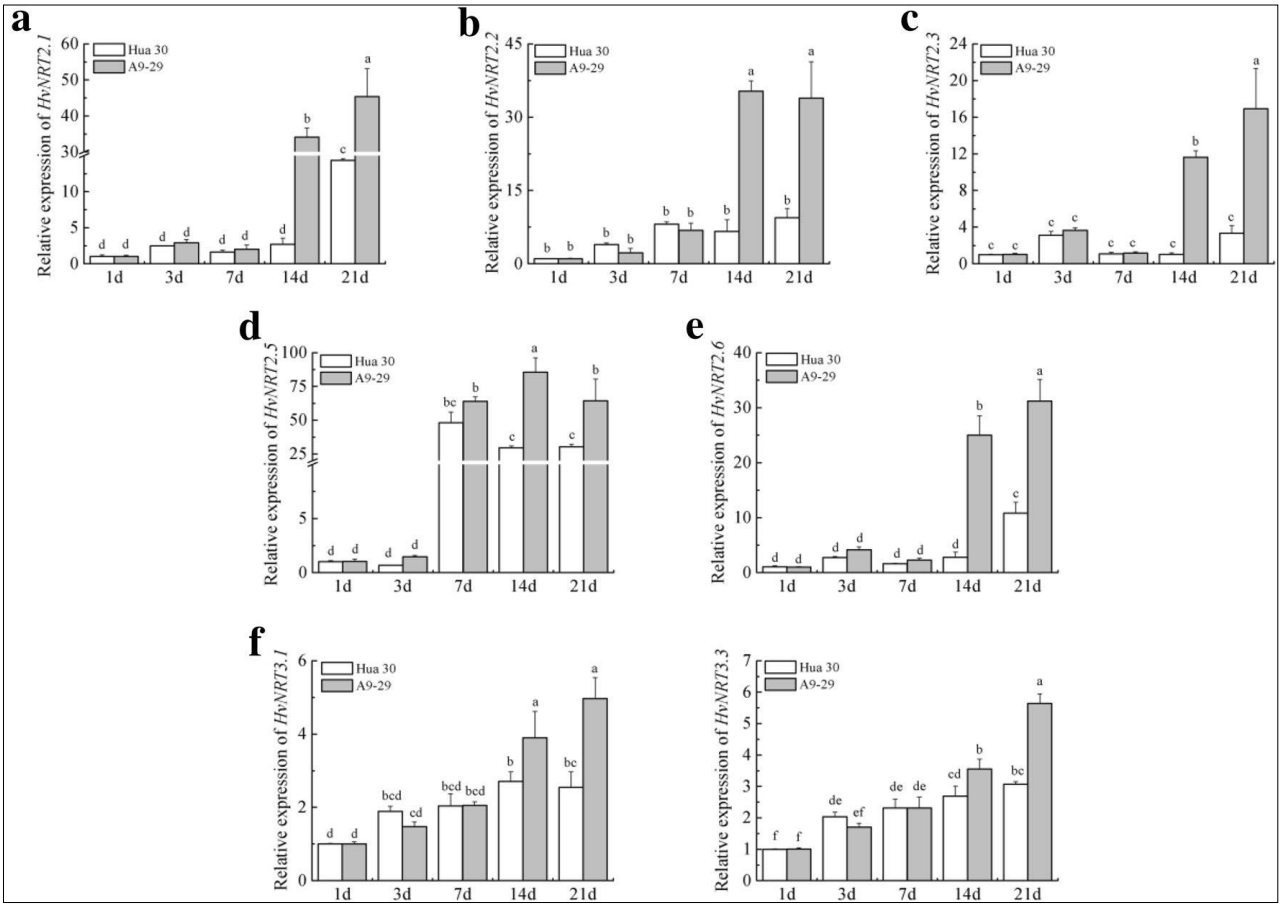

Fig 2: Relative expression of different HvNRTs under low and high nitrogen levels (after Gao, *et al*., 2021) [16]

The NUE of several spring-barley genotypes, grown under different environments showed dramatically genotypic and environmental variability, with low-N soil having greater NUE while yield decrease by 10%. With the improvement of modern breeding methods and intensive farming, the genetic uniformity of barley cultivars is increasingly enhanced,

losing many valuable alleles. Actually, cultivated barley shows more and more susceptibility to various abiotic and biotic stresses, including low soil fertility. In contrast, wild barley is rich in genetic diversity, containing the important genes or alleles for barley breeding. The modern barley originates from the wild barley of the Qinghai-Tibet Plateau of China and the Middle East "Fertile Bay" (Fertile Crescent). The earlier studies showed the wide genetic diversity of the wild barley in the Middle East, in particular for the tolerance to disease and abiotic stresses, such as drought, nitrogen starvation and salinity (Tables 2&3).

Table 2: Effect of different N levels on shoots NR, NiR, GS, GOGAT and GDH in four barley genotypes (after Decouard *et al*., 2022)

N level (mmol L^{-1})	Genotype (G)	NR (µgg^{-1} FW h^{-1})	NiR (mmol$L^{-1}NO_2^-mg^{-1}$ protein min^{-1})	GS (µmol g^{-1}FW h^{-1})	GOGAT(u mg^{-1} protein)	GDH (u mg^{-1} protein)
0	ZD9	20.73c	1.33f	62.43d	0.62f	0.79f
	XZ149	16.09ef	1.04g	51.94ef	0.49g	0.62g
	HXRL	13.81fg	0.92g	45.80fg	0.43g	0.55g
	XZ56	11.33g	0.74h	40.59g	0.35h	0.45h
0.2	ZD9	24.35b	2.20c	70.32bc	1.03c	1.32c
	XZ149	19.71cd	1.81d	59.80d	0.85d	1.08d
	HXRL	17.43de	1.50e	53.41e	0.70e	0.90e
	XZ56	12.49g	1.23f	43.03g	0.58f	0.74f
2.0	ZD9	30.94a	3.60a	88.24a	1.69a	2.15a
	XZ149	21.17c	2.33c	64.36cd	1.09c	1.40c
	HXRL	24.69b	2.60b	71.25b	1.22b	1.56b
	XZ56	16.27ef	1.68d	50.97ef	0.79d	1.01d

Table 3: Effect of different N levels on roots NR, NiR, GS, GOGAT and GDH in four barley genotypes

N level (mmol L^{-1})	Genotype (G)	NR (µgg^{-1} FW h^{-1})	NiR (mmol$L^{-1}NO_2^-mg^{-1}$ protein min^{-1})	GS (µmol g^{-1}FW h^{-1})	GOGAT (u mg^{-1} protein)	GDH (u mg^{-1} protein)
0	ZD9	13.49d	1.13g	28.23cd	0.55ef	0.68g
	XZ149	10.49fg	0.87h	23.33fgh	0.44g	0.52h
	HXRL	8.97gh	0.72hi	21.81ghi	0.32h	0.43hi
	XZ56	7.40h	0.68i	18.58i	0.30h	0.41i
0.2	ZD9	15.84bc	1.90d	33.85b	0.88c	1.14d
	XZ149	12.81de	1.56e	27.21cde	0.73d	0.94e
	HXRL	11.33ef	1.30f	25.43def	0.60e	0.78f
	XZ56	8.12h	1.06g	20.49hi	0.50fg	0.63g
2.0	ZD9	20.63a	3.19a	38.32a	1.45a	1.91a
	XZ149	14.59cd	2.07c	30.41bc	0.92c	1.24c
	HXRL	16.54b	2.42b	32.68b	1.15b	1.45b
	XZ56	10.07fg	1.45e	24.27efg	0.71d	0.87e

Investigation on the diversity of a North African barley genotype collection was carried out in terms of growth under limiting N (LN) or ample N (HN) supply and physiological traits including amino acid content in young seedlings. Researchers identified a Moroccan variety, Laanaceur, accumulating five times more lysine in its leaves than the others under both N nutritional regimes. Physiological characterization of the barley collection showed the genetic diversity of barley adaptation strategies to LN and highlighted a genotype x environment interaction. In all genotypes, N limitation resulted in biomass reduction, an increase in C concentration, and a higher resource allocation to the roots, indicating that this organ undergoes important adaptive metabolic activity. The most important are leaf nitrogen use efficiency (LNUE), root nitrogen use efficiency (RNUE), root nitrogen uptake efficiency (RNUpE), and leaf nitrogen uptake efficiency (LNUpE). Using LNUE as a target trait reflecting barley capacity to deal with N limitation, it was positively correlated with plant nitrogen uptake efficiency (PNUpE) and RNUpE. Based on the LNUE trait, researchers determined three classes showing high, moderate, or low tolerance to N limitation. The transcriptomic approach showed that signaling, ionic transport, immunity, and stress response were the major functions affected by N supply. A candidate gene encoding the HvNRT2.10 transporter was commonly up-regulated under LN. Genes encoding key enzymes required for lysine biosynthesis in plants, dihydrodipicolinate synthase (DHPS) and the catabolic enzyme, the bifunctional Lys-ketoglutarate reductase/ saccharopine dehydrogenase are up-regulated in Laanaceur and likely account for a hyper accumulation of lysine in this genotype (Decouard *et al*.,2021) [11] (Figs.3&4).

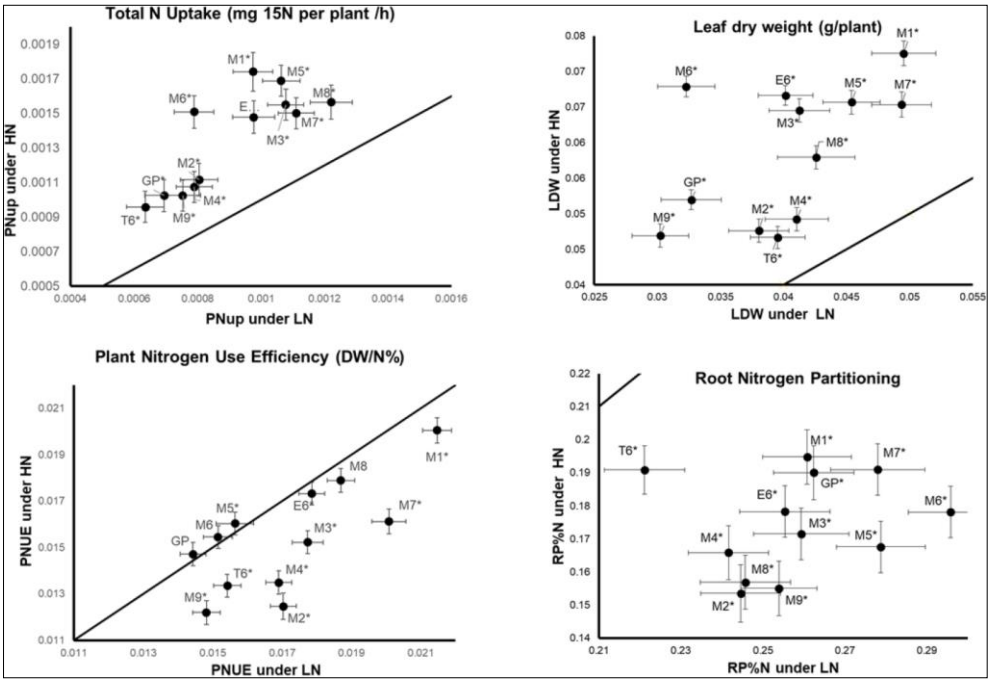

Fig 3: (A) Total plant N uptake (PNUpE). **(B)** Leaf dry weight (LDW). **(C)** Plant nitrogen use efficiency (PNUE). **(D)** Root nitrogen partitioning (RP%N). Mean values under HN are plotted against mean values under LN (after Decouard *et al*., 2021) [11]

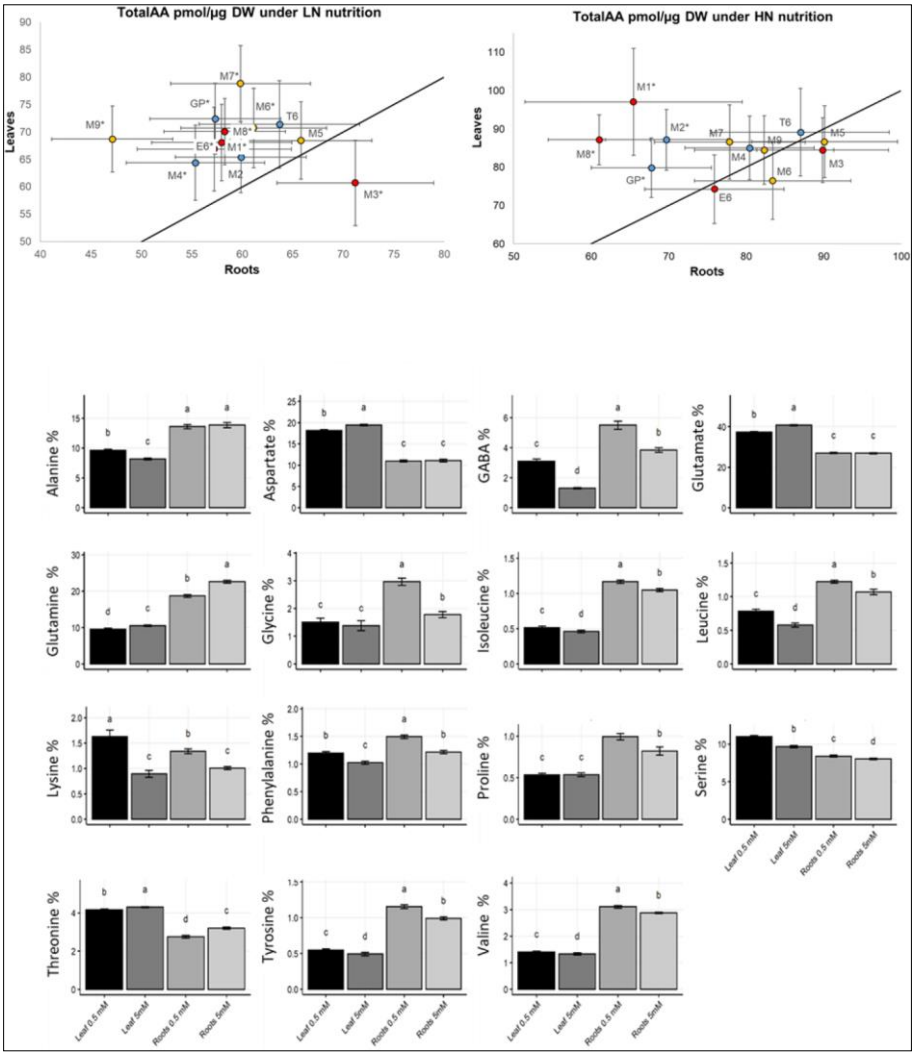

Fig 4: Investigations reveal the candidate metabolites involved in growth stimulation of barley seedlings after applying low-dose γ-radiation (60^0C) to seeds. Stimulating doses (5-20 Gy) provided a significant Fig.4. Amino acid distribution in barley leaves and roots under LN and HN (after Decouard *et al*., 2021) [11]

Increase in shoot length and biomass, while relatively high dose of 100 Gy led to significant inhibition of growth. Gas chromatography–mass spectrometry metabolomic analysis uncovered several compounds that includes molecules involved in nitrogen redistribution (arginine, glutamine, asparagine, and γ-aminobutyric acid) and stress-responsive metabolites, such as ascorbate, myo-inositol and its derivates, and free amino acids (L-serine, β-alanine, pipecolate, and GABA) (Volkova *et al*., 2020) [49] (Fig.5).

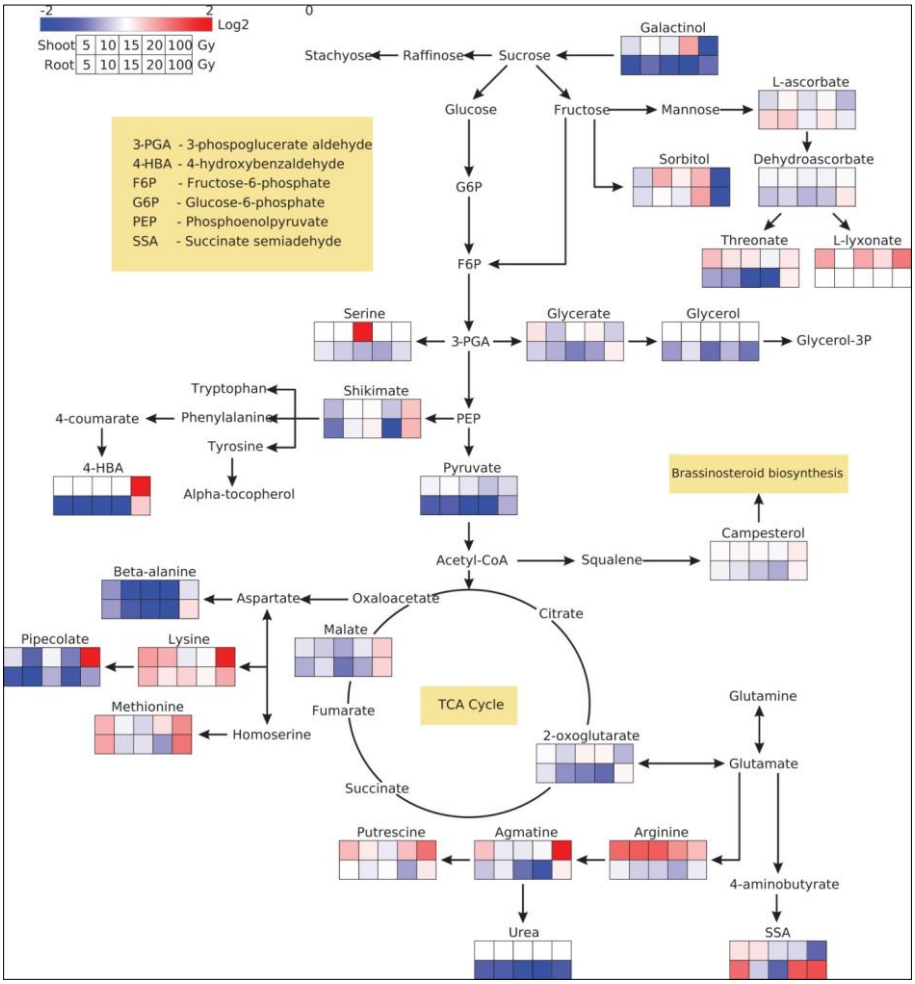

Fig 5: Schematic heat map reflecting interconnections between significant metabolites in roots and shoots

There are few studies on the mechanism of barley tolerance to low nitrogen at both the transcriptome and metabolomics levels. The nitrogen-efficient genotype (W26) and the nitrogen-sensitive genotype (W20) of barley were treated with low nitrogen (LN) for 3 days and18 days, and treated with resupplied nitrogen (RN) from 18 to 21 days. Later, the biomass and the nitrogen content were measured, and RNA-seq and metabolites were analyzed. The nitrogen use efficiency (NUE) of W26 and W20 treated with LN for 21 days was estimated by nitrogen content and dry weight, the values were 87.54% and 61.74%, respectively. As per transcriptome analysis, 7926 differentially expressed genes (DEGs) and 7537 DEGs were identified in the leaves of W26 and W20, respectively, and 6579 DEGs and 7128 DEGs were found in the roots of W26 and W20,respectively. After analysis of the metabolites, 458 differentially expressed metabolites (DAMs) and 425 DAMs were found in the leaves of W26 and W20, respectively, and 486 DAMs and 368 DAMs were found in the roots of W26 and W20, respectively. KEGG pathway analysis of DEGs and DAMs found that glutathione (GSH) metabolism was significantly enriched in the leaves of both W26 and W20. In leaves, GSH, amino acids, and amides were the main identified DAMs, while in roots, GSH, amino acids, and phenyl propanes were main DAMs. Finally, some nitrogen efficient candidate genes and metabolites were selected based on the results (Wang *et al*., 2023) [51] (Fig.6).

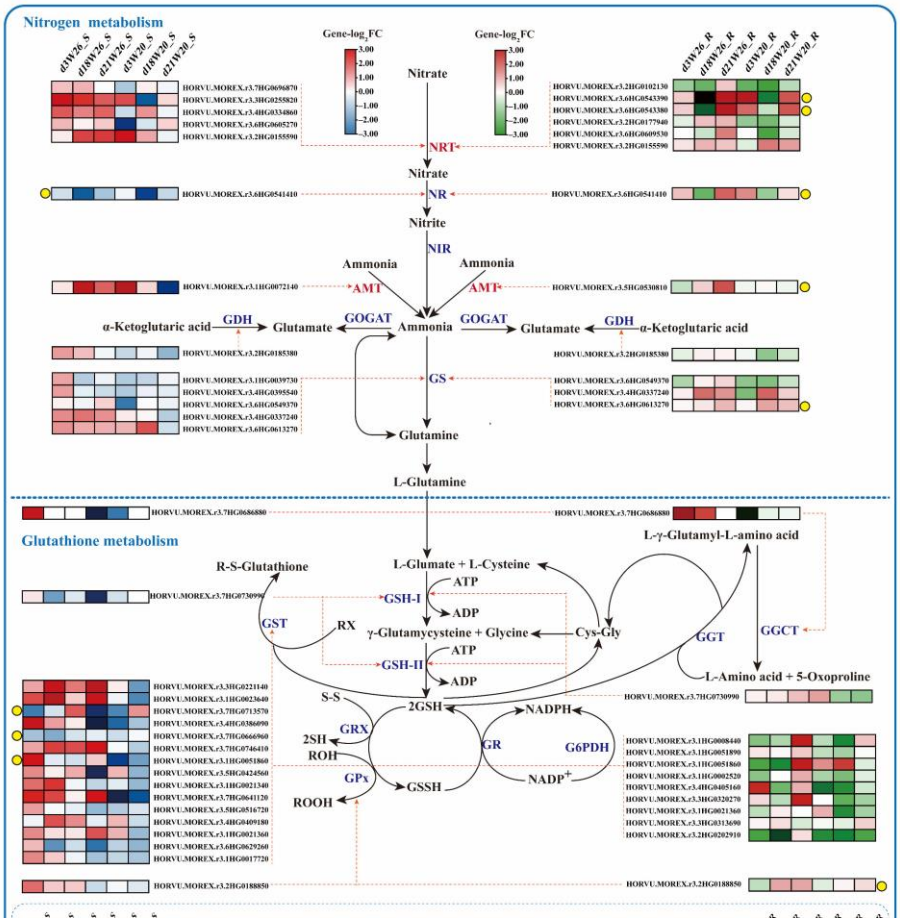

Fig 6: Nitrogen metabolism and GSH metabolism pathways

Quantitative trait loci (QTL) mapping in combination with marker-assisted selection (MAS) to track key regions of the chromosome that segregate for NUE is important. To achieve this goal, one of initial steps is to characterize the NUE-associated genes, then use the profiles of specific genes to combine plant physiology and genetics to improve plant performance. In a study, on the basis of genetic homology and expression analysis, barley candidate genes from a variety of families that exhibited potential roles in enhancing NUE were identified and mapped. Researchers then performed an analysis of QTLs associated with NUE in field trials and further analyzed their map-location data to narrow the search for these candidate genes. These results provide a novel insight on the identification of NUE genes and for the future prospects (Han *et al*., 2016) [21] (Table 4).

Table 4: Some nitrogen metabolism related genes of barley.

Heterotrimeric						
G-Protein	HvDEP1	5H	contig_37321	52.29	MLOC_52150L	1
	HvRGA1	7H	contig_52745	9. 06	MLOC_67224	8
	HvRGB1	4H	contig_65187	11.38	MLOC_74118	2
Mitogen-activate						
Kinase Kinase						
(MKK)	HvSMG1	6H	contig_1564374	78.4	MLOC_12915	2
	HvSMG2	5H	contig_134755	68.3	MLOC_4150	2
Sucrose non						
Fermenting-1						
Related Kinases						
(SnRK)	HvPKABA1	2H	contig_1561710	114.66	MLOC_11726	5
	HvPKABA2	2H	contig_5609	53.68	MLOC_69212	1
	HvPKABA3	4H	contig_160302	51.4	MLOC_22145	4
	HvPKABA4	5H	contig_127028	43.96	MLOC_3013	6
	HvPKABA5	2H	contig_46940	58.64	MLOC_62759	4
Early Nodulin						
Like Protein	HvEND93-1	7H	contig_1635653	23.8	MLOC_24054	1
	HvEND93-2	7H	contig_45347	43.59	MLOC_61290	1
	HvEND93-3	6H	contig_2552301	55.52	MLOC_39111	2

Amino Acid Biosynthesis Genes						
Glutamic-pyruvate						
Transaminase						
(GPT)	HvAlaAT1-1	1H	contig_51312	46.32	MLOC_66262L	1
	HvAlaAT1-1	2H	contig_37898	54.25	MLOC_52901	1
	HvAlaAT2-2	2H	contig_57179	58.78	MLOC_69931	3
	HvAlaAT1-1	5H	contig_138706	42.15	MLOC_7150	9
	HvAlaAT2-2	5H	contig_51539	49.89	MLOC_66427	5
GOGAT						
(GGT)	HvGGT1	1H	contig_45148	76.84	MLOC_57145	2
	HvGGT2	4H	contig_1577122	81.6	MLOC_17573	3
Asparagine						
Synthetase	HvASN1	4H	contig_274144	54.82	MLOC_44080	1
	HvASN4	5H	contig_47260	46.46	MLOC_63089	13
Asparaginase	HvASNase1	2H	contig_48445	91.01	MLOC_64169	12
	HvASNase2	2H	contig_51188	142.63	MLOC_66166	1
AAT	HvASP1	6H	contig_1573332	100.99	MLOC_16420	1
	HvASP2	1H	contig_156882	86.54	MLOC_14736	5
	HvASP3	7H	contig_2547742	76.47	MLOC_37080	3
	HvASP4	3H	contig_1566402	63.5	MLOC_13742	1
	HvASP5	6H	contig_90524	10.27	MLOC_80438	1
	HvASP6	5H	contig_40146	68.3	MLOC_55643	1
	HvASP7	3H	contig_159523	45.82	MLOC_21451	2
Asparagine						
Synthase	HvAS	5H	contig_9597	42.99	MLOC_81375	7
Glutamate						
Dehydrogenase						
NAD(P)H	HvGDH1	5H	contig_55763	139.24	MLOC_69020	4
	HvGDH2	3H	contig_499299	51.35	MLOC_65227	6
	HvGDH3	2H	contig_79282	81.8	MLOC_78233	3
	HvGDH4	3H	contig_2547948	52.03	MLOC_37189	1
Glutamine						
Synthetase	HvGS1	6H	contig_1562081	68.7	MLOC_11890	8
	HvGS2	4H	contig_1569958	60.69	MLOC_15134L	1
	HvGS3	2H	contig_38845	120.04	MLOC_54057	9
	HvGS4	4H	contig_46131	27.8	MLOC_62034L	3
	HvGS5	4H	contig_1573852	59.49	MLOC_16584L	1
Glutamate synthase						
(NADPH/Ferredoxin)	HvGOGAT1	3H	contig_1566054	51.62	MLOC_13604	3
	HvGOGAT2	2H	contig_5871	50.04	MLOC_70866	3
Glycolate oxidase						
(GOX)	HvGOX1	2H	contig_1572170	58.05	MLOC_16035	1
	HvGOX2	2H	contig_65448	58.64	MLOC_74253L	5
	HvGOX3	5H	contig_6695	136.59	MLOC_75010	4
	HvGOX4	2H	contig_52591	54.32	MLOC_67111L	8
	HvGOX5	N/A	contig_46080	N/A	MLOC_61991	3
Genes for N Assimilation						
Nitrate reductase	HvNR1	6H	contig_136596	82.36	MLOC_5716	2
	HvNR2	6H	contig_44311	10.27	MLOC_60358	1
Ferredoxin-nitrite						
Reductase	HvNiR1	6H	contig_273133	87.32	MLOC_43860	2
	HvNiR2	2H	contig_181042	43.97	MLOC_27159	1
Transcriptional Factors						
DNA-binding						

One Zinc Finger						
(DOF)	HvDOF1	5H	contig_327	75.88	MLOC_48629	1
	HvDOF2	2H	contig_160092	58.64	MLOC_21982	1
	HvDOF3	5H	contig_2548810	130.35	MLOC_37654	1
	HvDOF4	1H	contig_157123	17.28	MLOC_15655	1
	HvDOF5	7H	contig_49081	69.56	MLOC_64612	2
Nuclear factor						
Y (NFY)	HvNF-YB2.1	1H	contig_2547450	85.64	MLOC_36879	7
	HvNF-YB2.2	3H	contig_6163	98.65	MLOC_72428	5
	HvNF-YB2.3	2H	contig_42088	67.49	MLOC_57782	1
bHLH						
Transcriptional						
Factor	HvHLHm1	4H	contig_40514	59.63	MLOC_56065	3
	HvHLHm2	4H	contig_49250	36.35	MLOC_64735	2
	HvHLHm3	4H	contig_2546776	14.43	MLOC_36423	6
	HvHLHm4	4H	contig_53151	98.84	MLOC_67483	1
NAM, ATAF1,2, and CUC2 (NAC)						
	HvNAC1	4H	contig_54520	51.4 (O)	MLOC_68284	1
	HvNAC2	7H	contig_1707821	10.27	MLOC_25708	2
	HvNAC3	5H	contig_54346	80.34	MLOC_68185	2
	HvNAC4	5H	contig_25477871	50.07	MLOC_37104	2
	HvNAC5	7H	contig_38602	110.27	MLOC_53744	1
	HvNAM1	6H	contig_1574297	53.6	MLOC_16728L	3
	HvNAM2	2H	contig_141206	57.08	MLOC_8116	2
Aberrant panicle						
Organization	HvAPO1	N/A	contig_692	N/A	MLOC_75864	1
	HvFBX94	5H	contig_2547870	44.24	MLOC_37150	2
	HvFBX258	2H	contig_37898	54.25	MLOC_52901	1
Transporter Genes						
Nitrate transporter 2						
(high affinity)	HvNRT2.1	3H	contig_67100	55.81		
	HvNRT2.2	6H	contig_42664	13.67	MLOC_58437	1
	HvNRT2.3	6H	contig_42664	13.67	MLOC_58438	1
	HvNRT2.4	6H	contig_37664	13.67	MLOC_52621	1
	HvNRT2.5	6H	contig_49761	13.67	MLOC_65110	1
	HvNRT2.6	6H	contig_114886	13.52	MLOC_1673	1
	HvNRT2.7	7H	contig_58466	95.25	MLOC_70747	
NRT2 partner						
Protein (NAR2)	HvNAR2.1	6H	contig_127434	54.96	MLOC_3053	1
	HvNAR2.2	5H	contig_64422	155.56	MLOC_73802	1
	HvNAR2.3	6H	contig_44268	55.38	MLOC_60308	1
Ammonium						
Transporter	HvAMT1.1	6H	contig_240647	55.38	MLOC_33834	1
	HvAMT1.2	2H	contig_45766	67.49	MLOC_61695	1
Lysine histidine						
Transporter	HvLHT1	7H	contig_85053	52.27	MLOC_79443	1
	HvLHT2	7H	contig_1574246	70.54	MLOC_16705	3
	HvLHT3	7H	contig_38837	70.54	MLOC_54046	4
Other Genes						
Cytokinin oxidase/						
Dehydrogenase						
(CKX)	HvCKX1	3H	contig_95597	46.1	MLOC_8129	1
	HvCKX2	6H	contig_1569969	55.52	MLOC_1 5141	2
	HvCKX3	3H	contig_1573545	68.2	MLOC_16499	2
	HvCKX4	3H	contig_37260	135.62	MLOC_52060L	6

	HvCKX5	1H	contig_1560205	54.39	MLOC_11021	10
	HvCKX6	3H	contig_42846	45.82	MLOC_58639	1
	HvCKX7	2H	contig_37316	74.08	MLOC_52145	3
	HvCKX8	3H	contig_38743	47.1	MLOC_53923	1
Cytokinin						
Biosynthesis (IPT)	HvIPT1	1H	contig_1567227	37.6	MLOC_14093	1
	HvIPT2	2H	contig_71263	58.05	(O) MLOC_76403	6
	HvIPT3	3H	contig_37390	52.62	MLOC_52237L	1
	HvIPT4	3H	contig_37390	52.62	MLOC_52238	1
	HvIPT5	1H	contig_8161	107.29	MLOC_78718L	1
Cell wall						
Invertase	HvCIN1	4H	contig_49313	111.22	MLOC_64782	3
	HvCIN2	2H	contig_41327	58.78	MLOC_56998	4
	HvCIN3	1H	contig_136454	117.49	MLOC_5612	5
Stay-green						
Protein	HvSGR1	5H	contig_53834	98.13	MLOC_67884	3
Ferredoxin						
NADP (H)						
Reductase	HvFNR1	7H	contig_5804	81.63	MLOC_70480	1
	HvFNR2	5H	contig_138165	136.11	MLOC_6838	1
	HvFNR3	6H	contig_60084	3.75	MLOC_71570	2

Sulphur (S) is a component of diverse primary and secondary metabolites that play important roles in proper growth and development of plants. In cereals, a fraction of the nitrogen (N) accumulated in developing grains is guaranteed by amino acid remobilization from vegetative tissues, a contribution that becomes critical when soil nutrients are deficient. Glutamine synthetase (GS) and amino acid transporters (AAT) are key components involved in N assimilation and recycling. A study to evaluate the effect of S availability on the expressions of HvGS and several selected HvAAT genes in barley plants was shown by the phloem exudation rate of amino acids. Two independent experiments were designed to impose low S availability conditions to barley plants. Low S availability caused a decrease in the phloem exudation rate of amino acids as well as the gene expression of all the HvGS genes and five of the six HvAAT genes analyzed. The strong correlation found between the phloem amino acid exudation rate and HvGS1.1, HvGS1.2, HvAAP7, and HvProT1 gene expression may indicate the participation of these genes in the regulation of amino acid remobilization through the phloem (Veliz *et al*., 2017) [47].

Low Nitrogen Tolerance

The highest number of differentially expressed genes (8071) was with the highest mineral nitrogen rate. This number was 2.6 times higher than that for the group treated with a low nitrogen rate. The lowest number (500) was for the manure treatment group. Up regulated pathways in the mineral fertilizer treatment groups included biosynthesis of amino acids and ribosomal pathways. Down regulated pathways included starch and sucrose metabolism when mineral nitrogen was supplied at lower rates and carotenoid biosynthesis and phosphatidylinositol signaling at higher mineral nitrogen rates. The organic treatment group had the highest number of down regulated genes, with phenylpropanoid biosynthesis being the most significantly enriched pathway for these genes. Genes involved in starch and sucrose metabolism and plant-pathogen interaction pathways were enriched in the organic treatment group compared with the control treatment group receiving no nitrogen input (Esmaeilzadeh-Salestani *et al*., 2023) (Fig.7).

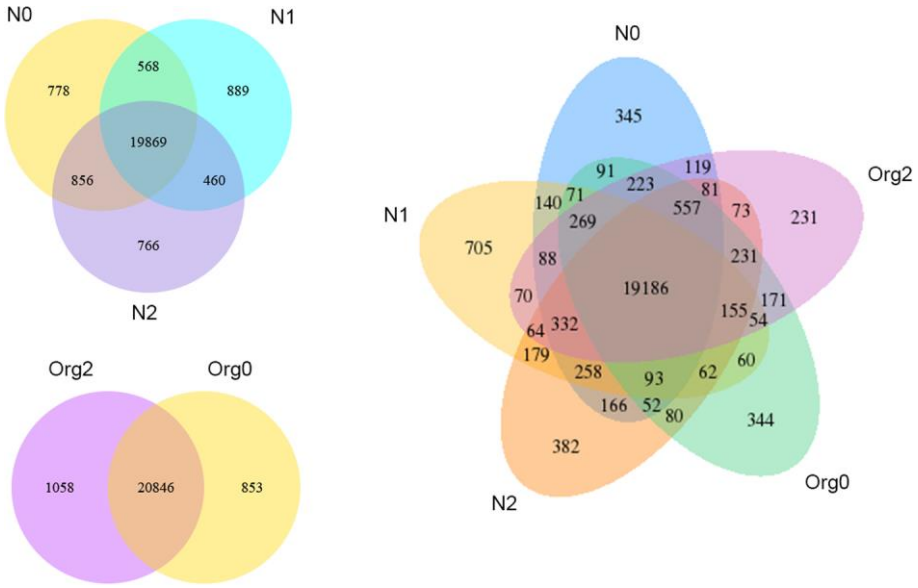

Fig 7: Venn diagrams of expressed genes in barley aboveground parts under different treatments

However, little is known about the differences among barley (*Hordeum vulgare* L.) genotypes in their responses to N starvation and subsequent N re-supply. Two barley genotypes, BI-04 (higher NUE) and BI-45 (lower NUE) were used to investigate N uptake and assimilation at seedling stage in response to N deprivation and re-supply at low (3.75 mM) and normal (7.5 mM) levels. Compared to the continues normal N supply, under N deprivation, both genotypes exhibited less total biomass and N accumulation, but had higher N uptake efficiency, with BI-04 having more biomass, N accumulation and nitrate reductase activity than BI-45. The higher nitrate reductase activity in roots of BI-04 versus BI-45 was associated with up-regulated HvNar1 gene expression under N deprivation condition. NUE of both genotypes was higher under low N re-supply than under normal N re-supply after N deprivation. In addition, glutamine synthetase activity in the two barley roots was higher under low N re-supply than under normal N re-supply, which was associated with the expression of HvGS1.1 and HvGS1.2 genes. Compared to the lower NUE genotype (BI-45), the higher NUE genotype (BI-04) under low N re-supply performed better in response to N stress, and may require relatively less N fertilizer application in production (Wang *et al*.,2023) [51].

N deficiency (ND) negatively affects leaf chlorosis, bud growth, and overall plant growth (Nasholm *et al*.,2009).This causes nutrient imbalance affecting several metabolic pathways including the increased production of reactive oxygen species (ROS)(Abrol *et al*.,1999; Rivero-Marcos *et al*.,2023). In rice, ND and the antioxidant system resulted in decreased light-harvesting capacity and increased thermal dissipation of absorbed energy(Huang *et al*.,1994).An increase in ROS imposes oxidative stress on plants, which utilize antioxidant enzymes, such as ascorbate oxidase (APX), peroxidases (POX), and catalase (CAT), to prevent excessive ROS accumulation (Agarwal *et al*., 2005) [3]. Wheat genotypes responded differentially to N supply in relation to leaf growth and photosynthesis as well as the maintenance of metabolic constituents (Sivasankar *et al*.,1998).

At the early vegetative stage of plant life, ND adversely influences crop yield, which cannot be offset by N application at later stages(Binder *et al*.,2000).Nitrogen fertilizer is applied to enhance crop yield because its availability strongly affects crop productivity. A significant amount of N contaminates ground and surface water and emits the greenhouse gas, nitrous oxide (Fowler *et al*.,2013).Thus, crop genotype development with improved nitrogen use efficiency (NUE) can aid in sustainable agriculture and high productivity under low-input conditions. NUE is a complex trait involving physiological, developmental, and environmental factors. This includes the plant's ability to absorb, transport, and remobilize N from the soil (Bi *et al*., 2009)(Fig.8).

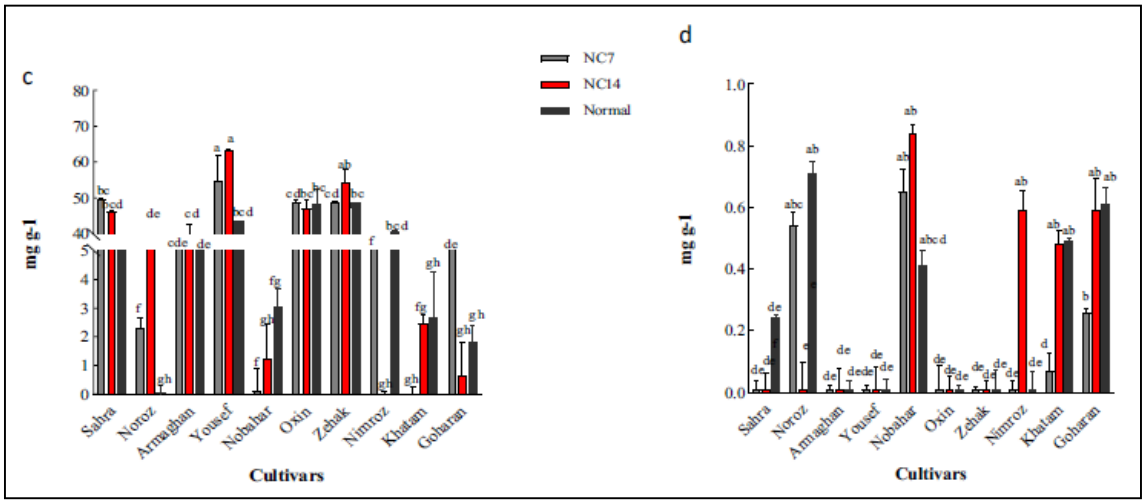

Fig 8: The effects of ND on 10 barley cultivars for total chlorophyll(**c**), carotenoid (**d**) at 7 and 14 days stress periods.NC7:7 days after ND application; NC14: 14 days after ND application(after Bi *et al*., 2009)

The current study was carried out to explore the potential of barley genotypes for higher NUE. A hydroponic experiment was conducted at seedling stage to compare the performance of four barley genotypes, ZD9 and XZ149 (with higher NUE) and HXRL and XZ56 (with lower NUE) in response to low (0.1 mM) and normal nitrogen (2 mM) levels. Under low N, all the genotypes expressed less number of tillers, decreased soluble proteins, chlorophyll and N concentrations in both roots and shoots, in comparison with normal N supply. However, significant differences were found among the genotypes. The genotypes with high NUE (ZD9 and XZ149) showed higher N concentration, increased number of tillers, improved chlorophyll and soluble proteins in both roots and shoots as compared to the inefficient ones (HXRL and XZ56). Furthermore, nitrate transporter gene (NRT2.1) showed higher expression under low N, both in roots and leaves of N efficient genotypes, as compared to the N inefficient ones. However, N assimilatory genes (GS1 and GS2) showed higher expression under normal and low N level, in leaves and roots respectively. It revealed that genotypes with higher NUE (ZD9 and XZ149) performed better under reduced N supply, and may require relatively less N fertilizer for normal growth and development, as compared to those with lower NUE also a time-specific expression pattern of studied genes, indicating the duration of low N stress (Shah *et al*.,2019).

In a study, four barley genotypes (two Tibetan wild and two cultivated), differing in N use efficiency (NUE), showed that higher N levels significantly increased the contents of other essential nutrients (P, K, Ca, Fe, Cu and Mn), and the increase was more for N-efficient genotypes (ZD9 and XZ149). The ultrastructure showed that chloroplast structure was severely damaged under low nitrogen, and the two high N efficient genotypes were relatively less affected. The activities of the five N metabolism related enzymes, i.e., nitrate reductase (NR), glutamine synthetase (GS), nitrite reductase (NiR), glutamate synthase (GOGAT) and glutamate dehydrogenase (GDH) all showed the substantial increase with the increased N level in the culture medium. However, the two N efficient genotypes showing more increase in comparison with the other two genotypes with relative N inefficiency (HXRL and XZ56). This suggests a huge difference exist in low N tolerance among barley genotypes, and improvement of some physiological traits (such as enzymes) could be helpful for increasing N utilization efficiency (Shah *et al*., 2017).

One of the most prevalent mechanisms of gene expression regulation in plants is microRNA-mediated silencing of target genes. Researchers identified 13 barley microRNAs and 2 microRNAs* that are nitrogen excess responsive. Four microRNAs respond only in root, eight microRNAs only in shoot and one displays broad response in roots and shoots. It was demonstrated that 2 microRNAs* are induced in barley shoot by nitrogen excess. For all microRNAs researchers identified putative target genes and confirmed microRNA-guided cleavage sites for ten out of thirteen mRNAs. None of the identified microRNAs or their target genes is known as nitrogen excess responsive. Analysis of expression pattern of thirteen target mRNAs and their cognate microRNAs showed expected correlations of their levels. The plant microRNAs analyzed are also known to respond to nitrogen deprivation and exhibit the opposite expression pattern when nitrogen excess/deficiency conditions are compared. Thus, they can be regarded as metabolic sensors of the regulation of nitrogen homeostasis in plants (Grabowska *et al*., 2020) (Table 5).

Table 5: The opposite expression pattern of selected conserved plant microRNAs responsive to N deficiency and N excess

microRNA family	Involvement under low N	Involvement under high N
	Plant tissue[a] Plant species	Plant tissue[a] Plant species
miRNA164	L(+),R(-),S(-) Maize [70,71]	R(+),S(+) Barley
miRNA169	R(-),S(-),L(-) Maize [71,72]	R(+),S(+) Barley
	R(-),SD(-) Arabidopsis [74]	
	R(-),S(-) Arabidopsis [48]	
	R(-),S(-) Soybean [75]	
miRNA171	R(+) Arabidopsis [73]	S(-) Barley
	R(+),S(+) Maize [71]	
	R(-),S(-) Soybean [75]	

miRNA319	R(-) Maize [70]	S(+) Barley
	R(-),S(-) Soybean [75]	
miRNA393	R(+) Maize [71]	S(-) Barley
miRNA396	R(-) Maize [71]	R(+) Barley
miRNA398	R(-),SD(-) Arabidopsis [73,74]	S(+) Barley
	L(-),S(-) Maize [71,73]	
	R(-),S(-) Soybean [75]	
miRNA399	L(-),R(-) Maize [70,71]	R(+) Barley
	R(-) Arabidopsis [73]	
miRNA408	L(-),R(-) Maize [71,76]	S(+) Barley
	R(+) Arabidopsis [73]	
	R(-),S(-) Soybean [75]	
miRNA528	R(-),S(-),L(-) Maize [71,76]	S(+) Barley
miRNA827	R(-) Arabidopsis [73]	R(+) Barley
	L(-),R(-) Maize [70,71]	
miRNA6177	No data	S(+) Barley

[a]Plant tissue:L, leaf;R, root;S, shoot;SD, seedling; (+),upregulated; (-), downregulated

An effect of nitrogen rates (0.0 g, 1.0 g, 2.0 g N per pot) on NRA in leaves of spring barley (cv. Kompakt) was investigated in a pot experiment. Plants were grown under optimum moisture regime and drought stress was induced during the growth stages of tillering, shooting and earing. Before and after respective stress period plants were grown under optimal water regime. NRA was significantly higher under optimal water regime than in drought stress conditions. Nitrogen application alleviated adverse effects of drought stress on the yields of grain; the rate of 1 g N per pot increased the grain yield of plants,stressed during tillering, 3.73 times compared to unfertilized and stressed treatment. When the stress was induced during shooting or earing grain yields declined by over 50% compared to optimal water regime; when compared with stressed and unfertilized treatment, the rate of 1 g N however increased yield by 29% (stress at shooting) and 55% (stress at earing). NRA values were significantly higher under optimum water regime than under stress conditions as well as when fertilized with nitrogen compared to unfertilized control both under optimum water regime and drought stress (Krcek *et al*.,2008).

To study remobilization and grain yield of barley genotypes, two separate experiments were conducted at the Agricultural and Natural Resources Research Station of Miandoab during the years of 2014–2016 as a split plot based on randomized complete block design with three replications. The treatments included 5 genotypes and four nitrogen fertilizer levels (control or without fertilizer, 50, 100 and 150 kg ha^{-1} nitrogen (N) fertilizer. The maximum remobilization was obtained at 0 and 50 kg N levels. N application increased non-significantly the remobilization under water deficit stress. The highest (1.22 g.m^{-2}) and lowest (0.91 g.m^{-2}) remobilization were recorded in 100 kg ha^{-1} N and control. Bahman genotype, as well as Karoon and NK1272 genotypes had the higher remobilization under well irrigation and water deficit, respectively. The highest remobilization to grain was related to 100 and 150 kg ha^{-1} N. The comparison of N application levels showed that the highest current photosynthesis contribution for seed yield was in 150 kg ha^{-1} N. Under water deficit, it was allocated to 50 kg ha^{-1}. The greater grain yield in tolerant genotypes under water deficit was due to remobilization of carbohydrates from shoot to grain. Thus, it seems that selection of genotypes with higher translocation of dry matter and reserve assimilate during grain filling under water deficit is suitable cultivars with high grain yield(Table 6)(Ghaderi *et al*.,2023).

Table 6: Prospective genes related to low water and low nitrogen in barley

Cell wall invertase	HvCIN1 4H	contig_49313	111.22	MLOC_64782	3
	HvCIN2 2H	contig_41327	58.78	MLOC_56998	4
	HvCIN3 1H	contig_136454	117.49	MLOC_5612	5
Stay-green protein	HvSGR1 5H	contig_53834	98.13	MLOC_67884	3
Ferredoxin					
NADP(H) reductase	HvFNR1 7H	contig_58048	1.63	MLOC_70480	1
	HvFNR2 5H	contig_138165	136.11	MLOC_6838	1
	HvFNR3 6H	contig_60084	3.75	MLOC_71570	2

Two to five haplotypes in each gene were discovered in a set of 190 varieties. 33 SNP markers allowed the genotyping of all these barley varieties consisting of spring and winter types. Furthermore, these markers could be mapped in several doubled haploid populations. Cluster analysis based on haplotypes revealed a more uniform pattern of the spring barleys as compared to the winter barleys. Based on linear model approaches, associations to several malting and kernel quality traits including soluble N and protein were identified (Matthis *et al*.,2013).

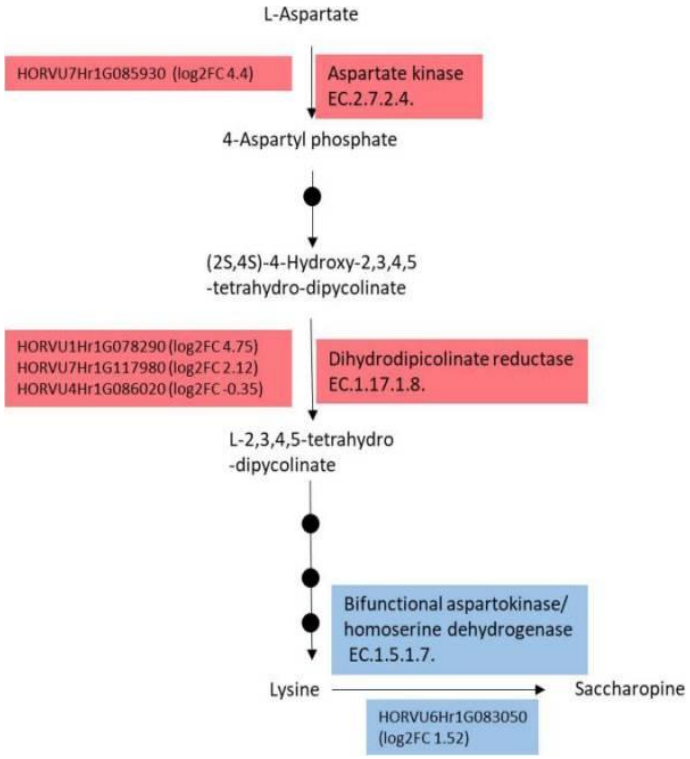

Fig 9: Simplified Lysine biosynthesis and catabolism pathways were found to be differentially expressed in M4 compared to GP and M5. Red and blue boxes correspond to biosynthesis and catabolism of lysine, respectively. Black dots represent intermediate enzymatic steps that were omitted for simplification (after Decouard *et al*., 2021) [11]

Salinity Tolerance

The interaction between salinity and nitrogen metabolism has been investigated in two barley landraces, one tolerant ("100/1B") and one susceptible to salinity ("Barley medenine") from the Middle East and North Africa (MENA) region. Barley plants were exposed to 50 mM NaCl for 7 days; then, salinity was increased to 150 mM NaCl in the presence (10 mM) or limitation (1 mM) of ammonium as a nitrogen source. Upon salinity, "100/1B" was shown to support N assimilation by enhancing the glutamine synthetase (GS) and glutamine oxoglutarate aminotransferase (GOGAT) cycle under high N, and the stimulation of the glutamate dehydrogenase (GDH) pathway under low N treatment. In "Barley medenine", salinity reduced the GS/GOGAT cycle, and increased GDH activity. Upon salinity, Heat Shock Proteins 70 and PEPC remained unchanged in "100/1B", while they decreased in "Barley medenine". The tolerance degree is a determining factor in enzymes' occurrence and regulation: exposed to salinity, "100/1B" rapidly increased APX and PEPC activities, while this was delayed in "Barley medenine". Salinity increased cyt-G6PDH levels in "100/1B", while "Barley medenine" showed a decrease in G6PDH isoforms. Correlation analyses confirm GOGAT was related to G6PDH; GDH and APX with PEPC in"100/1B" under moderate salinity; severe salinity correlated GDH with G6PDH and PEPC. In "Barley medenine" under salinity, GOGAT was correlated with G6PDH, while APX showed a relation with PEPC(Ben Azaiez *et al*.,2022).

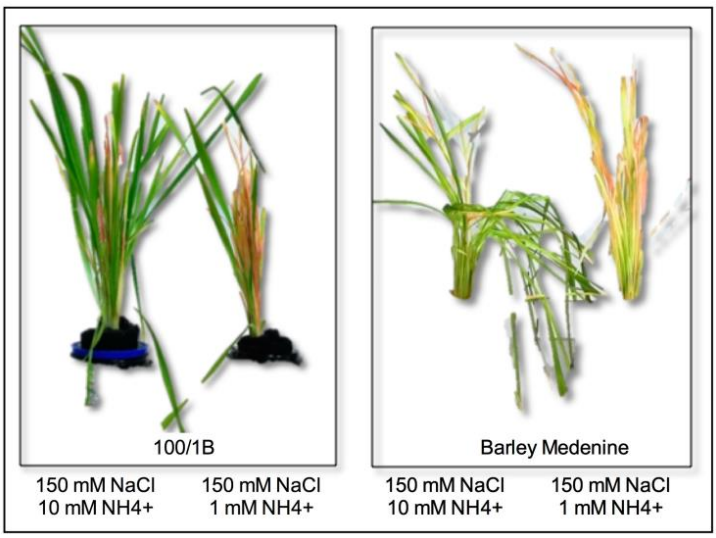

Fig 10: Effects of salinity and N supply on "100/1B" and "Barley medenine" leaves

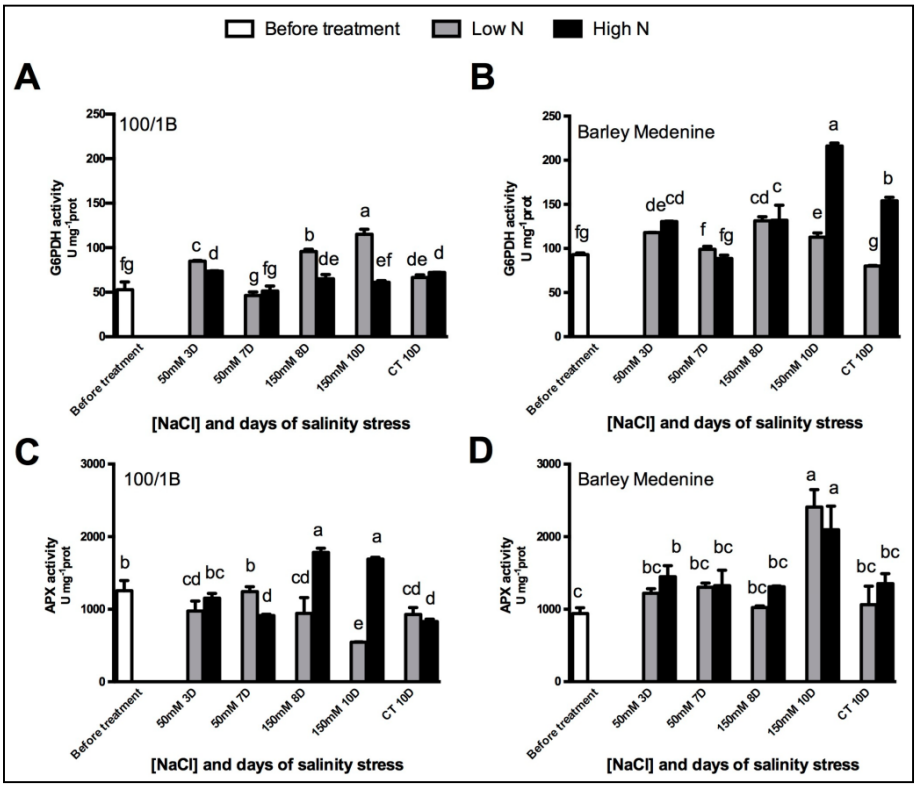

Fig 11: Effects of salinity and N concentration on G6PDH and APX enzymatic activities in barley plants growth in hydroponic system

Conclusion

Good amount of works on genetics had been made available by now. However, researchers may concentrate on the genetic aspects of genotype-low nitrogen-environment interactions. In breeding and biotechnology the prospect of including wild varieties and mutant characteristics and gene pool are to be considered in further research.

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