

## Polysomes and RNA Analysis of High Lysine Barley Mutant Notch-2 and its Parent NP113

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Barley mutant Notch-2, isolated by ethyl methyl sulphate (EMS) treatment of NP 113 seeds has a uniform depression on dorsal side (Bansal 1970). Reduction in yield in the mutant is mainly due to reduction in starch accumulation towards later stage of development (Mehta *et al.* 1979). The mutant also has low level of hordein (Bansal *et al.* 1977) and lesser number of hordein polypeptides (Bhattacharya *et al.* 1986) as compared to the parent. In an attempt to understand the differential developmental pattern of the mutant, analysis of polysomes and RNA from developing endosperms of NP 113 and Notch-2 was carried out and reported here.

Barely (*Hordeum vulgare* L.) seeds of high lysine mutant Notch-2 and its parent NP 113 were collected at 17 and 24 days after anthesis (DAA) from the field grown crop. Endosperms were dissected out manually and polysomes were isolated as described by Luthe and Peterson (1977) with certain modifications which included 0.5 mM PMSF and 100  $\mu\text{g mL}^{-1}$  heparin and Nonidet P-40 in place of sodium deoxycholate in polysome extraction buffer. Post polysomal pellets were obtained as described by Nichols and Welder (1983). Separation of polyribosomes and monosomes on sucrose density gradient was carried out following the method of Mifflin and Fox (1977). Polysomal and post polysomal RNA were isolated from the polysomal and post polysomal pellets respectively using phenol-chloroform extraction method. RNA concentration was determined spectrophotometrically considering one  $A_{260}$  unit equivalent to 40  $\mu\text{g}$ .

Proportion of polysomes was greater than monosomes in both NP 113 and Notch-2, while the trend was reverse at 24 DAA. This shows that both NP 113 and Notch-2 maintain high level of monosomes and very low level of polysomes at later

stage of endosperm development. Similar results have been reported earlier in pea cotyledons (Poulson & Beevers 1973). The high level of monosomes in at later stage of development seems to be required for early protein synthesis during germination (Yadav *et al.* 1990).

At both the stages, NP 113 had higher proportion of polysomes than Notch-2 endosperms. It was also seen that  $A_{260}/A_{280}$  ratio of total polysomes was lower in Notch-2 endosperms than in NP 113 indicating greater proportion of proteins associated with polysomes in Notch-2-endosperms. This might be due to formation of ribonucleoprotein complexes resulting into translational control of certain protein in developing Notch-2-endosperms. Sen and Mehta (1980) have reported that decreased protein content in the mutant is neither due to limitation of amino acids nor due to any of the key nitrogen assimilating enzymes. It is, therefore, most likely that lower level of polysomes in the mutant might be responsible for its decreased protein content.

Polysomal RNA accounted for over 89% of the total in both the varieties and was more in NP 113 than in Notch-2. Similarly it was 3.2 and 2.1 times greater in the parent compared to the mutant on

Table 1 Per cent distribution of polysomes from developing endosperms of NP 113 and Notch-2 barely grains

Development stage	Variety	Poly-some area	Mono-some area	Poly-some percent	Mono-some percent
17DAA	NP 113	1961	707	73.5	26.5
	Notch-2	1429	632	69.3	20.7
24DAA	NP 113	725	1234	37.0	63.0
	Notch-2	452	1275	26.2	73.8

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**Table 2** RNA content ( $\mu\text{g } 100^{-1}$  endosperms) in developing endosperms

Develop mental stage	Variety	Weight of endosperms (g)	Poly somal RNA ( $\mu\text{g}$ )	Post omal RNA ( $\mu\text{g}$ )	Total RNA ( $\mu\text{g}$ )
17DAA	NP 113	5.58	831	39	870
	Notch-2	5.31	260	34	294
24 DAA	NP 113	7.11	1116	47	1163
	Notch-2	6.30	529	35	564

per endosperm basis at the respective stages. This might have resulted in lower hordein content in the mutant as zelooted by Bansal et al. (1977). This is supported by earlier study in which dot blot hybridization of RNA with hordein probe has show A lower hordein mRN in Notch-2 relative to NP 113 (Tyagi et al. 1992)

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Table 1 Per cent distribution of polyomes from developing endosperms of NP 113 and Notch-2 barley genues

Develop-mental stage	Variety	Poly-some sites	Mono-some sites	Poly-some percent	Mono-some percent
17DAA	NP 113	1961	707	73.2	26.2
	Notch-2	1429	632	69.3	20.7
24DAA	NP 113	722	1294	37.0	63.0
	Notch-2	422	1272	26.2	73.8

the method of Mifflin and Fox (1977). Polyosomal and post polyosomal RNA were isolated from the phenol-chloroform extraction method. RNA concentration was determined spectrophotometrically considering one A<sub>260</sub> unit equivalent to 40  $\mu\text{g}$ . Proportion of polyomes was greater than monosomes in both NP 113 and Notch-2 while the trend was reverse at 24DAA. This shows that both NP 113 and Notch-2 maintain high level of monosomes and very low level of polyomes at later