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The nature of the leucine requirement of the barley mutant *Xan*-b^{61}

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SUMMARY

Contrary to previous reports, evidence has been presented which indicates that *Xan*-b^{61}, a *Xantha* mutant of barley, does not have an auxotrophic requirement for leucine. It has been shown that this mutant, when grown under appropriate conditions in the absence of leucine, will synthesize leucine and will continue to grow for an extended period of time.

Since the first report (Walles, 1963), that the chlorosis of *Xan*-c^{23}, an induced chloroplast mutant of barley, could be alleviated by the presence of substrate levels of leucine in the growth medium, several reviews have appeared in which similar *Xantha* mutants have been stated to show a requirement for this amino acid for normal growth (Walles, 1968; Nelson, 1967; von Wettstein & Eriksson, 1963). Recent work in this laboratory with *Xan*-b^{61}, a mutant requiring leucine for normal growth (von Wettstein & Eriksson, 1963), has been undertaken to clarify the nature of this requirement for this mutant. The experimental approach used was first, to determine whether leucine supplementation did induce normal chlorophyll production and growth in the mutant; second, to ascertain whether the mutant possessed the ability to synthesize leucine when grown on simple inorganic media in the light; and third, to test whether supplementation with sucrose might replace leucine and overcome the genetic block.

Seeds segregating the *Xan*- b^{61} mutant were grown in the light under aseptic conditions using a modified Hoagland–Went nutrient solution described by Walles (1963). After segregation, the seedlings were maintained in this nutrient medium, hereafter termed basal medium, or were transferred to the basal medium supplemented with either 0·2% L-leucine (Walles, 1963) or 2% sucrose. Throughout the experimental period conditions of strict asepsis were maintained. After suitable intervals, the plants were harvested and the fresh weight and chlorophyll content of the leaf material determined. Tissue protein and soluble amino acids were estimated by means of an automatic amino acid analyser.

Leucine, present in the growth medium at the levels employed by Walles (1963), alleviated the expression of chlorosis in the mutant but the resultant growth of both the mutant and the green segregant was abnormal and depressed (Table 1). Thus even at the high concentration employed, leucine did not completely overcome the genetic block since normal chlorophyll production was not restored.

The ability of the mutant to synthesize leucine was demonstrated by a comparison of the analytical data for this amino acid present in the segregating seed with that in the mutant seedling after seven days growth on the basal medium (Table 2). Much of the

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increase in leucine could be accounted for in the protein of the mutant and green segregant tissue but in the former there was a marked increase in the total soluble amino acids and soluble leucine. Accumulations of this nature have been reported for various types of genetical and physiological chloroses (DeKock & Morrison, 1958).

Table 1. *The fresh weight (g per shoot) and chlorophyll content (the absorbance of the ethanolic extract at 663 nm per shoot per ml) of leaf material of* Xan-*b*^{61} *and green segregants grown on basal medium in the presence and absence of 0·2% leucine for 11 days*

	Xan-b^{61}		Green segregant	
	F.W.	Chlorophyll	F.W.	Chlorophyll
Basal medium	0·269	3·50	0·321	8·75
Basal medium plus leucine	0·274	5·47	0·309	9·37

Table 2. *The total leucine content of the segregating seed and seedlings of* Xan-*b*^{61} *and green segregants grown on basal medium for 7 days and the total soluble amino acid content and the leucine component of the leaf material of these seedlings (µmoles per seed or seedling)*

		Seedling	
	Seed	*Xan*-b^{61}	Green segregant
Total leucine	3·3	4·3	4·8
		Shoot	
Soluble leucine	—	0·12	0·019*
Total soluble amino acids	—	4·6	1·5*

* The variety Bonus in this instance was employed as a control.

Table 3. *The fresh weight (g per shoot), chlorophyll content (the absorbance of the ethanolic extract at 663 nm per shoot per ml) and leucine content (µmoles per shoot) of the leaf material of* Xan-*b*^{61} *and green segregants grown on basal medium in the presence and absence of 2% sucrose for 11 days*

	Xan-b^{61}			Green segregant		
	F.W.	Chlorophyll	Leucine	F.W.	Chlorophyll	Leucine
Basal medium	0·162	2·13	1·66	0·249	15·92	3·27
Basal medium plus sucrose	0·580	6·71	3·43	0·482	33·09	8·70

When grown on the basal medium for 11 days the fresh weight and chlorophyll content of the mutant was much lower than the control (Table 3). Supplementation of the medium with 2% sucrose resulted in considerable increases in the fresh weight and chlorophyll content of both the mutant and control. Under these conditions the mutant exhibited less chlorosis and its growth and appearance resembled that of the green segregant grown on the basal medium. No abnormal growth effects were observed. Further, both the mutant and green segregant when grown on the sucrose supplemented medium contained more leucine than the corresponding material grown on the basal medium (Table 3). The leucine content of the sucrose grown mutant was higher than the green segregant grown on the basal medium.

Under the conditions employed in this work it is evident that the mutant plant has the ability to synthesize leucine and, if presented with favourable conditions in the absence of leucine, will grow normally for prolonged periods. From the evidence presented it is concluded that *Xan*-b^{61} has no auxotrophic requirement for this amino acid and it would be incorrect to equate a requirement of this type with the classical concept of auxotrophic growth. Similar work is in progress using other mutants in this series.

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