CHAPTER 4

RESULTS

4.1 ANALYSIS OF RAW MATERIALS

4.1.1 Proximate Analysis of Pearl Millet Varieties

The proximate composition of the two pearl millet varieties used in this research work is shown in Table 6.

TABLE 6.- Proximate composition (%) of the two pearl millet varieties used in this investigation

Pearl Millet Variety	Moisture	Protein (N x 6.25)	Fat	Ash	Carbohydrate	Fibre (by difference)
SDMV 89004	9.4	10.6 (11.7)[a]	5.8 (6.4)[a]	1.3 (1.4)[a]	71.0 (78.4)[a]	1.9 (2.1)[a]
SDMV 91018	12.0	9.9 (11.3)[b]	5.6 (6.4)[a]	2.6 (2.9)[b]	66.3 (75.3)[b]	3.6 (4.1)[b]

Mean values with different letters in each column are significantly different from each other ($p < 0.001$).

Results in brackets are on dry basis.

Results are mean of three replicates.

Variety SDMV 89004 had significantly higher protein and carbohydrate contents than SDMV 91018. Variety SDMV 91018 had significantly higher ash and fibre contents. Fat was similar in both varieties.

4.1.2 Enzyme Susceptibility of Carbohydrate, Amylose-Amylopectin Ratio and Gelatinisation Temperature Range of Pearl Millet Varieties

The percentage of the TCES, the amylose-amylopectin ratio as well as the gelatinisation temperature range of pearl millet varieties are shown in Table 7.

TABLE 7.- Enzyme susceptibility of carbohydrate, amylose-amylopectin ratio and gelatinisation temperature range of pearl millet varieties used in this investigation

Pearl Millet Variety	% of Total Carbohydrate Enzyme-Susceptible	Amylose-Amylopectin Ratio (%)	Gelatinisation Temperature Range (°C)
SDMV 89004	10.6[a]	19.0/81.0[a]	63.5 - 70.9[a]
SDMV 91018	10.1[a]	20.4/79.6[a]	65.8 - 73.4[b]

Mean values with different letters in each column are significantly different from each other ($p < 0.001$).
Results are mean of three replicates.

There was no significant difference between the percentage of the TCES between the two pearl millet varieties. Variety did not have any effect on the amylose-amylopectin ratio. SDMV 91018 had a significantly higher gelatinisation temperature than SDMV 89004.

4.1.3 Antinutritional Factors in Pearl Millet Varieties

The percentages of phytic acid and total polyphenols are shown in Table 8.

TABLE 8.- Phytic acid and polyphenol content of the two pearl millet varieties used in this investigation

Pearl Millet Variety	Phytic Acid (%)	Total Polyphenols (%)
SDMV 89004	0.22 (0.24)[a]	0.08 (0.09)[a]
SDMV 91018	0.24 (0.27)[b]	0.10 (0.11)[a]

Mean values with different letters in each column are significantly different from each other ($p < 0.001$).

Results in brackets are on dry basis.

Results are mean of three replicates.

The percentage of phytic acid of the variety SDMV 89004 was significantly lower than SDMV 91018.

After submiting the two millet varieties to the Chlorox bleach test no black kernels were observed, i.e. none of the grains were of the high tannin type.

There was no significant difference between the content of total polyphenols in the two pearl millet varieties. The level of polyphenols in both pearl millet varieties was low.

4.1.4 Endosperm Texture of Pearl Millet Varieties

After cutting the grains in two lengthwise halves using a sharp disposable scalpel, the texture of the endosperm of the pearl millet of variety SDMV 89004 was found to be softer compared with the SDMV 91018. Varieties SDMV 89004 and SDMV 91018 had an endosperm textures of 2.5 and 3.2, respectively.

4.1.5 Germinative Energy (GE) and Germinative Vigour (GV) of Pearl Millet Varieties

The percentage Germinative Energy and Germinative Vigour is shown in Table 9.

TABLE 9.- Germinative Energy and Germinative Vigour of the two pearl millet varieties used in this investigation

Pearl Millet Variety	Germinative Energy (%)			Germinative Vigour (%)		
	24h	48h	72h	24h	48h	72h
SDMV 89004	94.0[a]	97.9[a]	99.6[a]	92.0[a]	96.2[a]	98.0[a]
SDMV 91018	89.9[b]	93.1[b]	95.5[b]	86.9[b]	91.0[b]	92.5[b]

Mean values with different letters in each column are significantly different from each other ($p < 0.001$).

Results are mean of three replicates.

Both pearl millet varieties had high Germinative Vigour and high Germinative Energy. Variety SDMV 89004 had significantly higher Germinative Energy and Germinative Vigour than SDMV 91018.

4.1.6 Water Uptake of Pearl Millet Varieties

The effects of steeping time and temperature as well as variety on water uptake of the two pearl millet varieties are shown in Table 10.

Water uptake was significantly affected ($p < 0.001$) by time and temperature of steeping. In general, variety did not have any effect on water uptake ($p > 0.05$). Water uptake increased with steeping time for both pearl millet varieties. Water uptake increased with an increase in steeping temperature for both pearl millet varieties. The lowest percentage of water uptake 14.7% was observed at 6 h steeping time at 20 °C with SDMV 91018. The highest water uptake 25.3% was recorded at 10 h steeping time at 35 °C with variety SDMV 89004.

TABLE 10.- Water uptake of the two pearl millet varieties at various steeping temperatures and times

Steeping Temperature (°C)	Steeping Time (h)	Water Uptake (%) Variety SDMV 89004	SDMV 91018
20	6	15.9[a]	14.7[a]
	8	18.9[b]	16.1[b]
	10	19.0[b]	16.2[b]
25	6	17.0[a]	16.7[a]
	8	19.9[b]	19.5[b]
	10	19.7[b]	19.6[b]
30	6	19.1[a]	18.8[a]
	8	21.7[b]	21.2[b]
	10	22.3[c]	21.3[b]
35	6	22.2[a]	21.4[a]
	8	24.8[b]	21.8[b]
	10	25.3[c]	23.2[c]

Means values with different letters in each block are significantly different from each other ($p < 0.001$).

Results are mean of three replicates.

4.2 PEARL MILLET ENDOSPERM MODIFICATION DURING GERMINATION

Figure 8 shows pearl millet malts of variety SDMV 89004, germinated for 5 days, with long roots and shoots.

The pearl millet grains of both varieties were tear-shaped and the germ was large in proportion to the rest of the kernel. The kernel contained floury and horny endosperm portions (Figure 9).

In the non-germinated grains, the floury opaque endosperm contained spherical (round) or polygonal starch granules and many air spaces (Figure 10). There appeared to be no protein bodies in the opaque endosperm. The horny endosperm was devoid of air spaces and contained polygonal starch granules (Figure 11).

Initially (24 h), modification of starch and the protein matrix could be observed in the floury endosperm adjacent to the scutellum epithelium (Figure 12). At the beginning of germination, degradation appeared as pin holes in the starch granules and as germination progressed these holes became bigger and appeared to coalesce (Figure 13). As germination progressed, degradation was observed at further distances from the germ in the direction of the horny endosperm. The proportion of highly degraded starch granules decreased from the proximal to the distal end of the grain. There was not much modification on the cell walls, aleurone layer and horny endosperm during germination (Figure 9). Protein body degradation occurred to a lesser extent than starch granule degradation (Figure 14).

The pearl millet grains, which were germinated for longer periods, showed a seedling with long roots and shoots and almost completely empty starchy endosperm. In this research, both pearl millet varieties showed up signs of germination (chitting) between 8 and 10 h of germination.

Figure 8.- Pearl millet malts of variety SDMV 89004 germinated for 5 days (average mass of each kernel: 8.9 mg; average length of the roots and shoots: 15-20 mm)

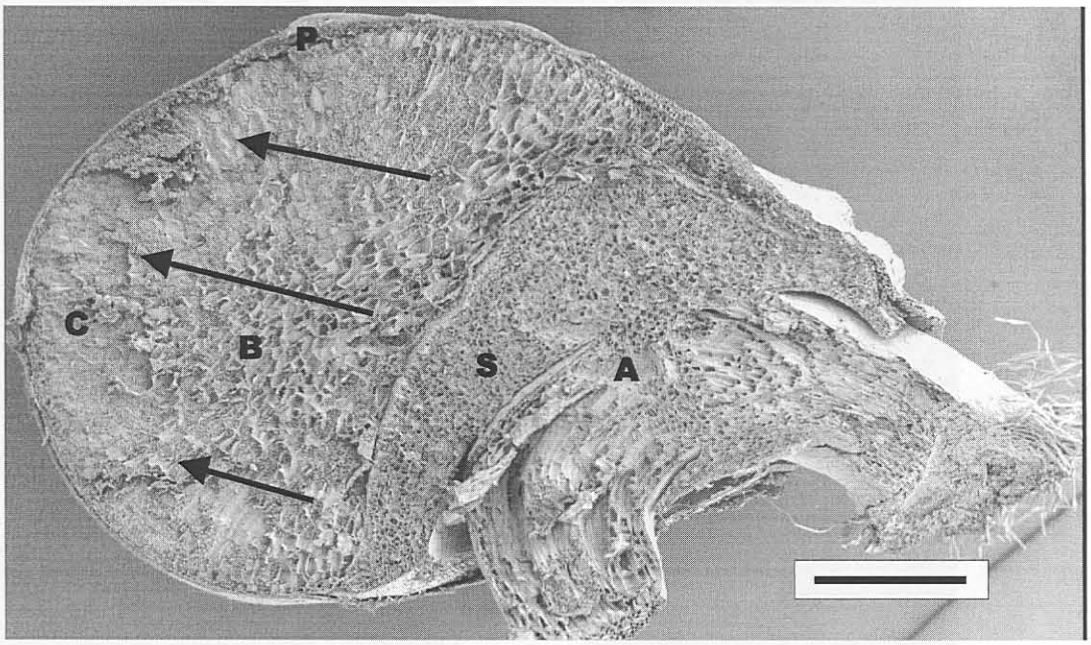

Figure 9.- Longitudinal section of pearl millet malt of variety SDMV 91018 germinated for 5 days showing the large germ in proportion to the rest of the kernel and the wave of modification (arrows) Germ; B- Floury endosperm; C- Horny endosperm; P- Pericarp; S- scutellum) (Bar = 600 μm)

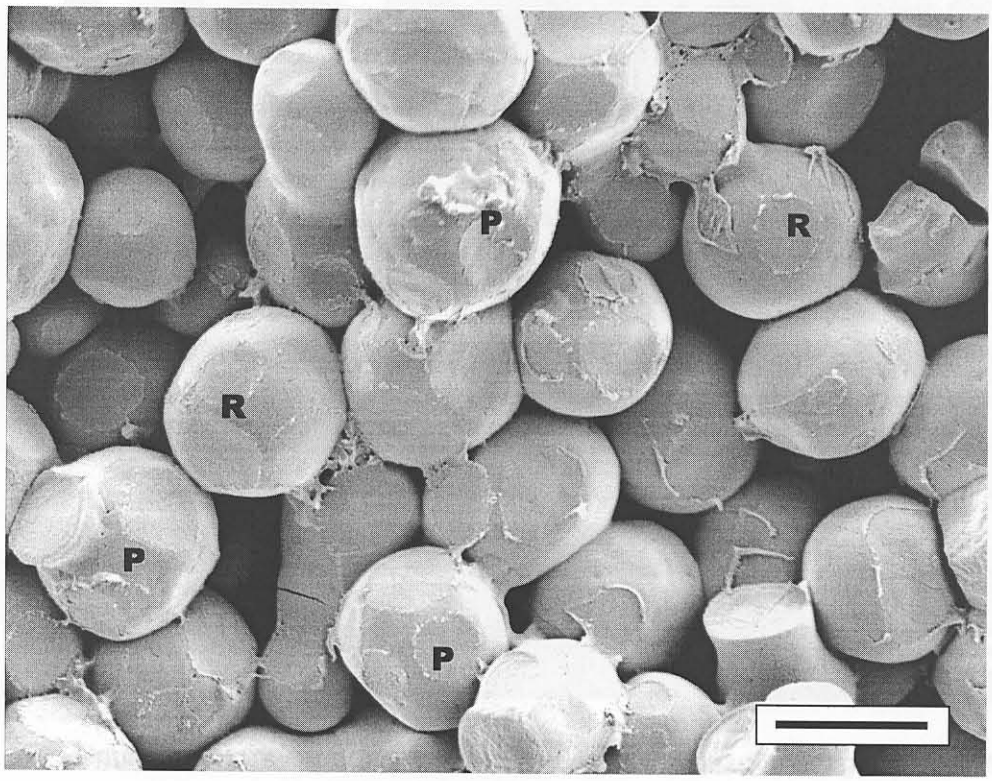

Figure 10.- Floury endosperm of non-germinated pearl millet of SDMV 89004 variety showing both rounded (R) and polygonal (P) starch granules (Bar = 8 μm)

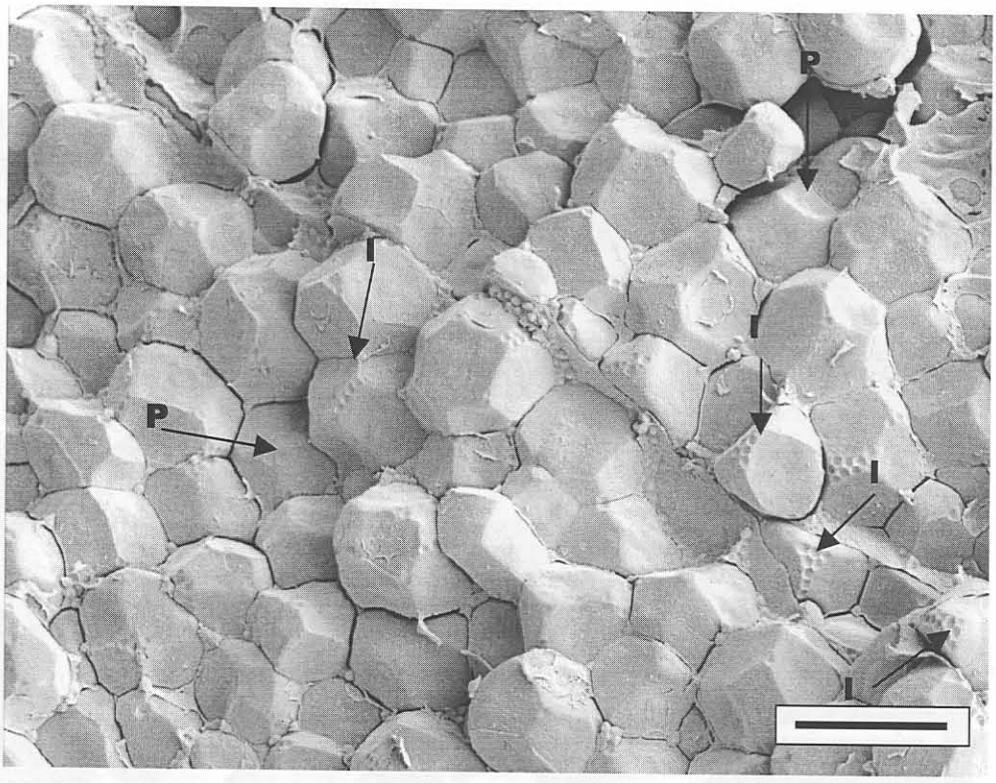

Figure 11.- Horny endosperm of non-germinated pearl millet grain of SDMV 91018 variety showing polygonal (P) starch granules and imprints (I) of protein bodies (Bar = 9 μm)

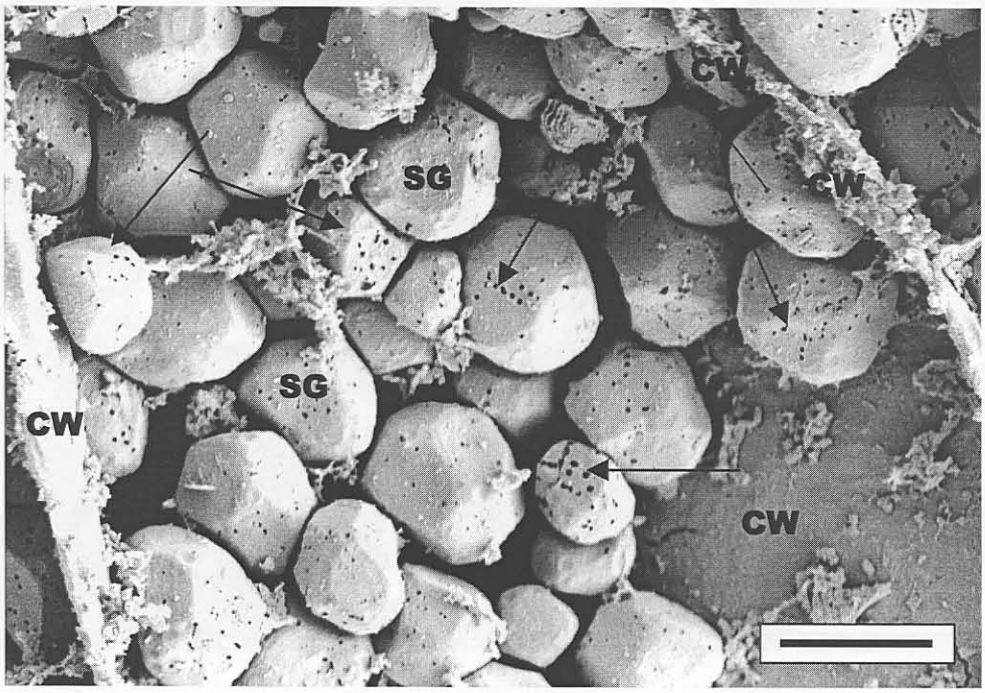

Figure 12.- Floury endosperm adjacent to scutellum epithelium of pearl millet of variety SDMV 89004 after 24 h of germination showing pin holes (arrows) in the starch granules (CW- Cell wall; SG- Starch ganule) (Bar = 8 μm)

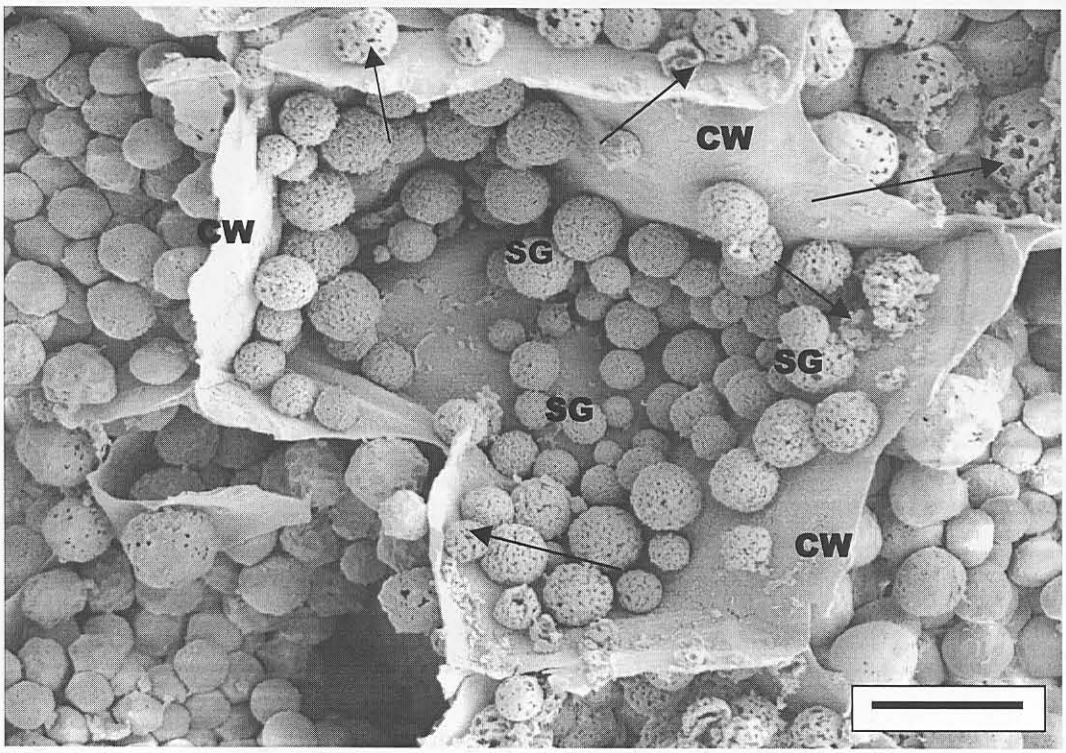

Figure 13.- Floury endosperm adjacent to scutellar epithelium of pearl millet of SDMV 89004 variety after 72 h of germination showing highly degraded starch granules (arrows) and intact cell wall (CW- Cell wall; SG- Starch granules) (Bar = 13 μm)

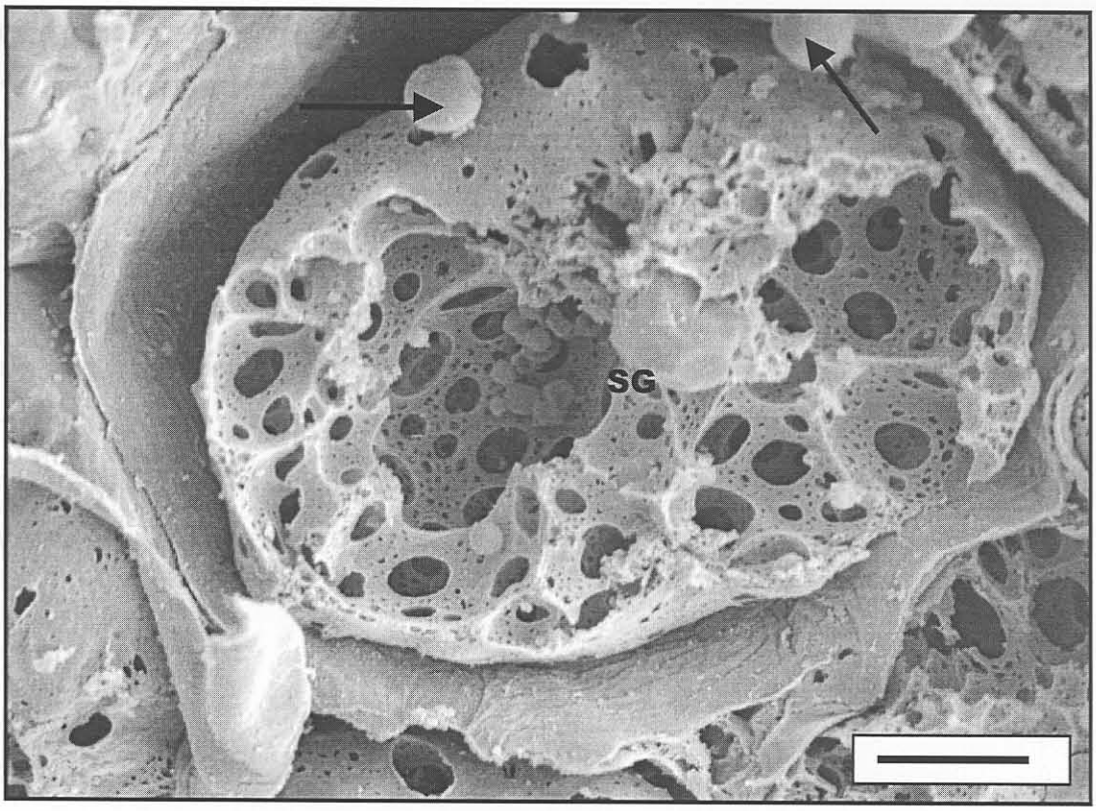

Figure 14.- Scanning electron micrograph of completely degraded starch granule in modified pearl millet malt of variety SDMV 89004 germinated for 48 h (Arrows- Protein body;SG- Starch granule) (Bar = 3 μm)

4.3 BREWING QUALITY ANALYSES OF MALTS

Pearl millet malts steeped for 8 h with a cycle of 2 h wet and 2 h dry air rest and germinated at 20 °, 25 °, 30 °, and 35 °C, medium watering treatment were analysed in terms of brewing quality, i.e. water uptake during germination, root and shoot growth during germination, Diastatic Power (DP), α- and β-amylase activity, free α-amino nitrogen (FAN), malt extract and malting loss. At the optimum germination temperature of 25 °C, high and low watering treatments, malts were analysed in terms of DP, total and soluble β-amylase activity (Betamyl assay), FAN, malt extract and malting loss. Total and soluble β-amylase activity (Betamyl assay) and malt extract of peal millet malts were compared to barley and sorghum malts standard.

4.3.1 Water uptake during steeping

The effects of steeping temperature and variety on water uptake during steeping of the two pearl millet varieties, at 8 h steeping time, are shown in Table 10 and described under 4.1.6.

4.3.2 Root and Shoot growth during germination

The effects of germination time, temperature and variety on root and shoot growth of the two pearl millet varieties are shown in Figure 15.

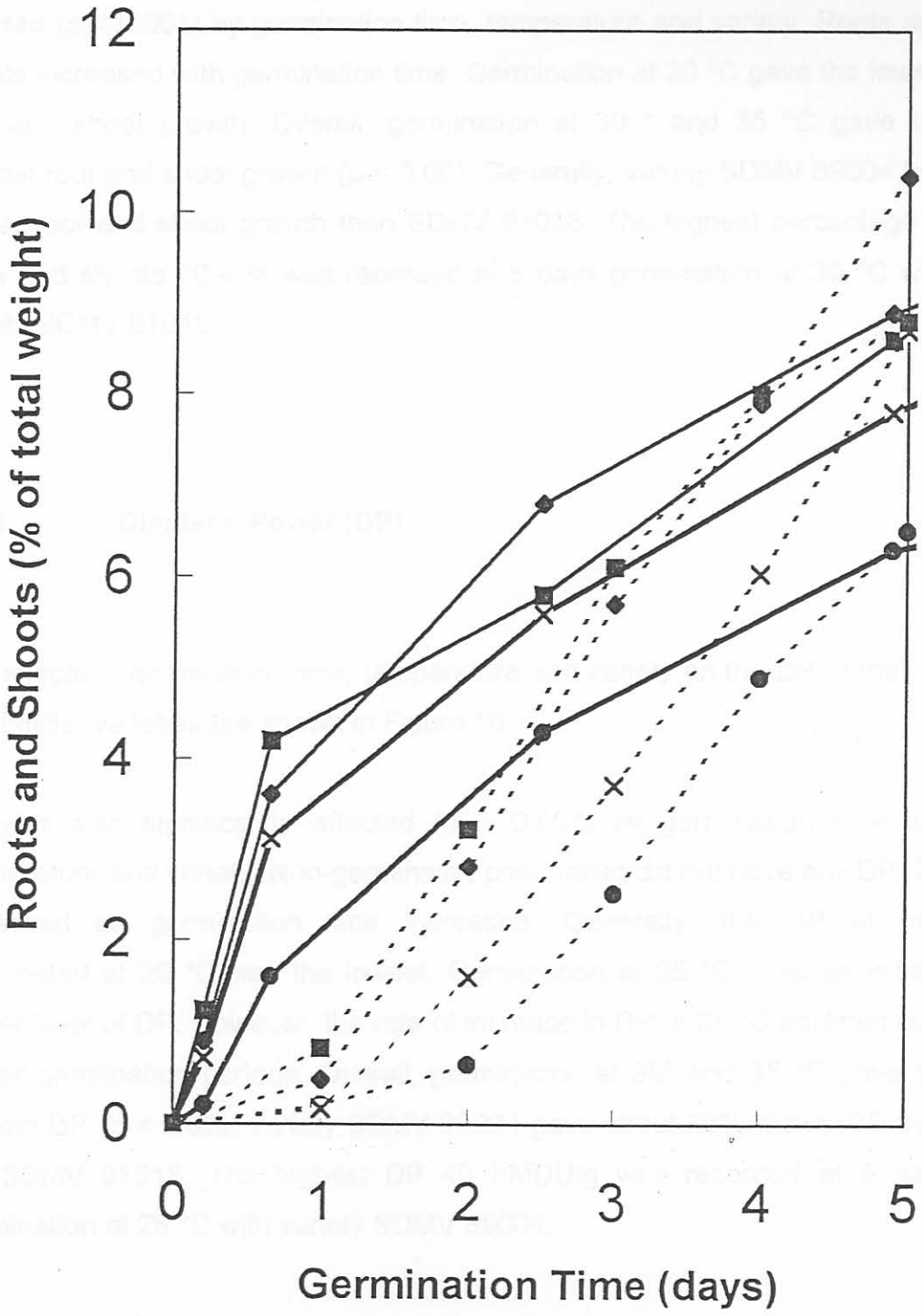

Figure 15.- Effects of germination time, temperature and variety on root and shoot growth of pearl millet (variety SDMV 89004 (–) and variety SDMV 91018 (--); (•- 20 °C; ×- 25 °C; ♦- 30 °C; ■- 35 °C)

The percentage of roots and shoots in pearl millet malt was significantly affected ($p < 0.001$) by germination time, temperature and variety. Roots and shoots increased with germination time. Germination at 20 °C gave the lowest root and shoot growth. Overall, germination at 30 ° and 35 °C gave the highest root and shoot growth ($p < 0.05$). Generally, variety SDMV 89004 had higher root and shoot growth than SDMV 91018. The highest percentage of roots and shoots 10.4 % was recorded at 5 days germination at 30 °C with variety SDMV 91018.

4.3.3 Diastatic Power (DP)

The effects of germination time, temperature and variety on the DP of the two pearl millet varieties are shown in Figure 16.

DP was also significantly affected ($p < 0.001$) by germination time and temperature and variety. Non-germinated pearl millet did not have any DP. DP increased as germination time increased. Generally, the DP of malt germinated at 20 °C was the lowest. Germination at 35 °C gave an initially higher level of DP. However, the rate of increase in DP at 35 °C declined over longer germination periods. Overall, germination at 30° and 35 °C gave the highest DP ($p < 0.05$). Variety SDMV 89004 gave about 30% higher DP than the SDMV 91018. The highest DP 40 PMDU/g was recorded at 5 days germination at 25 °C with variety SDMV 89004.

The effects of watering treatment, germination time and variety on DP of the two pearl millet varieties at the germination temperature of 25 °C are shown in Figure 17.

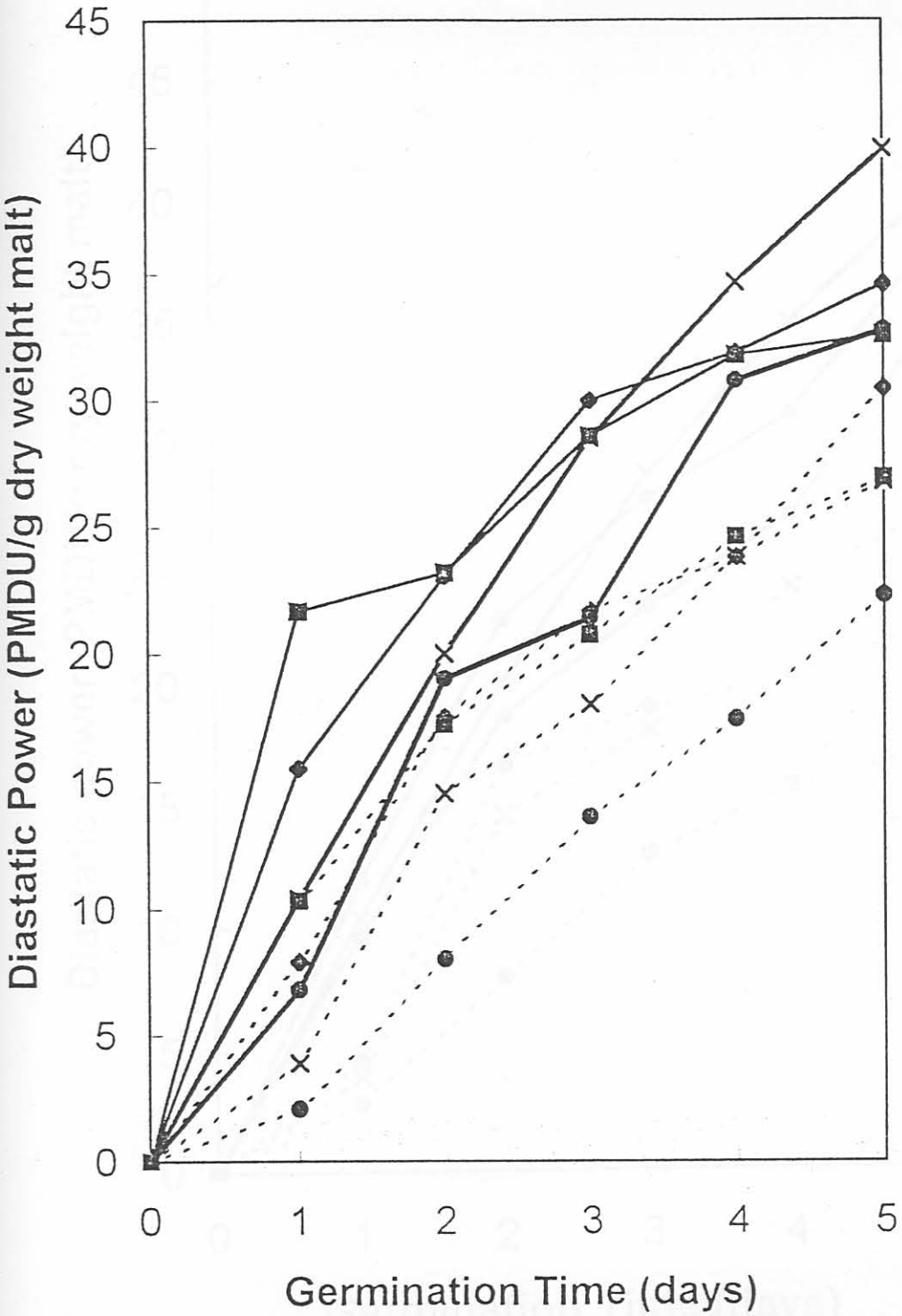

Figure 16.- Effects of germination time, temperature and variety on the DP of pearl millet (variety SDMV 89004 (–) and variety SDMV 91018 (--); (•-20 °C; ×- 25 °C; ♦- 30 °C; ■- 35 °C)

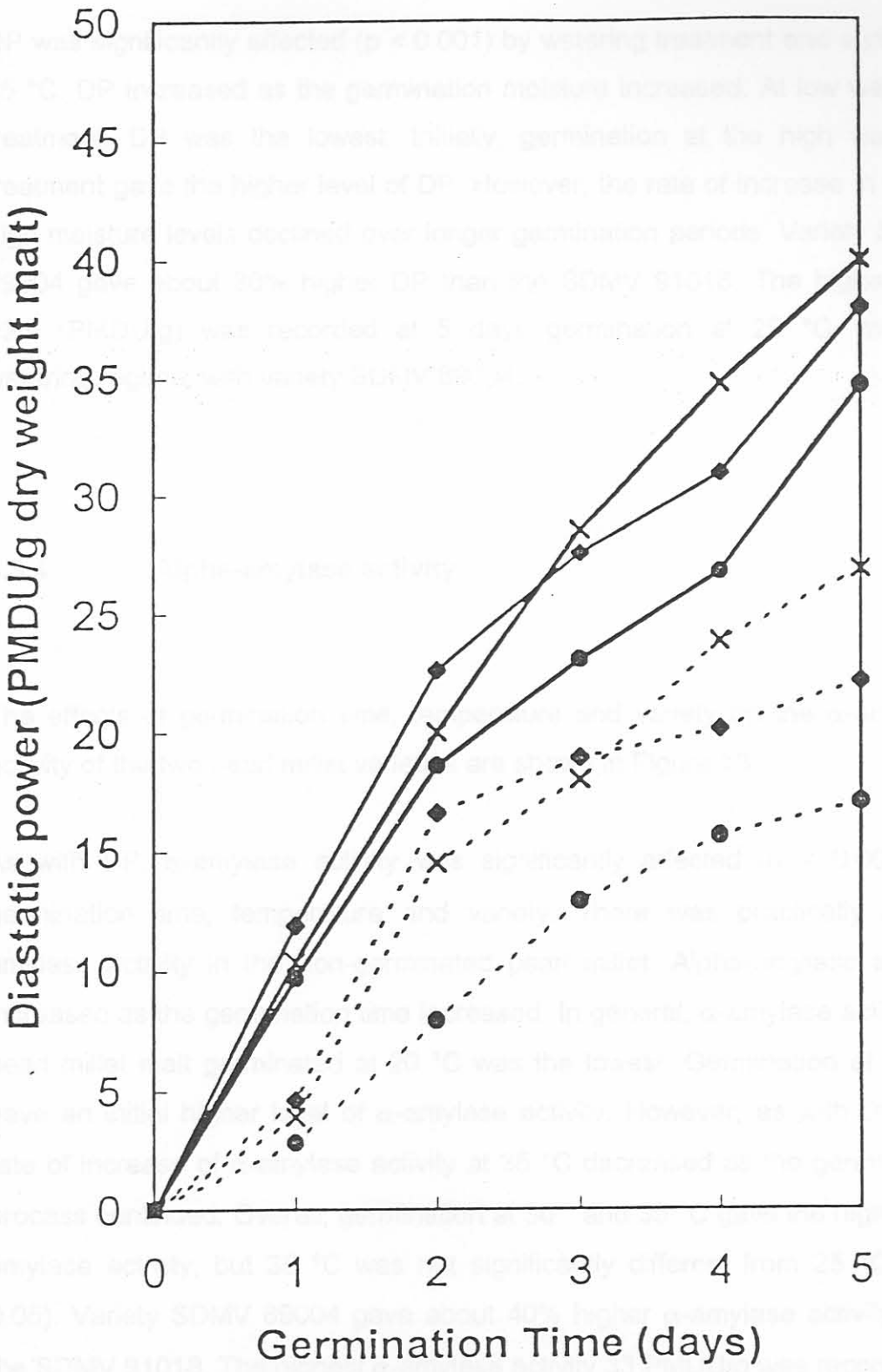

Figure 17.- Effects of germination time, watering treatment and variety on the DP of pearl millet at 25 °C (variety SDMV 89004(–) and variety SDMV 91018 (--) at various watering treatments (•- Low watering; ×- Medium watering; ♦- High watering)

DP was significantly affected ($p < 0.001$) by watering treatment and variety at 25 °C. DP increased as the germination moisture increased. At low watering treatment, DP was the lowest. Initially, germination at the high watering treatment gave the higher level of DP. However, the rate of increase in DP at high moisture levels declined over longer germination periods. Variety SDMV 89004 gave about 30% higher DP than the SDMV 91018. The highest DP 39.9 (PMDU/g) was recorded at 5 days germination at 25 °C, medium watering regime, with variety SDMV 89004.

4.3.4 Alpha-amylase activity

The effects of germination time, temperature and variety on the α-amylase activity of the two pearl millet varieties are shown in Figure 18.

As with DP, α-amylase activity was significantly affected ($p < 0.001$) by germination time, temperature and variety. There was practically no α-amylase activity in the non-germinated pearl millet. Alpha-amylase activity increased as the germination time increased. In general, α-amylase activity of pearl millet malt germinated at 20 °C was the lowest. Germination at 35 °C gave an initial higher level of α-amylase activity. However, as with DP, the rate of increase of α-amylase activity at 35 °C decreased as the germination process continued. Overall, germination at 30 ° and 35 °C gave the highest α-amylase activity, but 35 °C was not significantly different from 25 °C ($p > 0.05$). Variety SDMV 89004 gave about 40% higher α-amylase activity than the SDMV 91018. The highest α-amylase activity 33 PMDU/g was recorded at 5 days germination at 25°C with variety SDMV 89004.

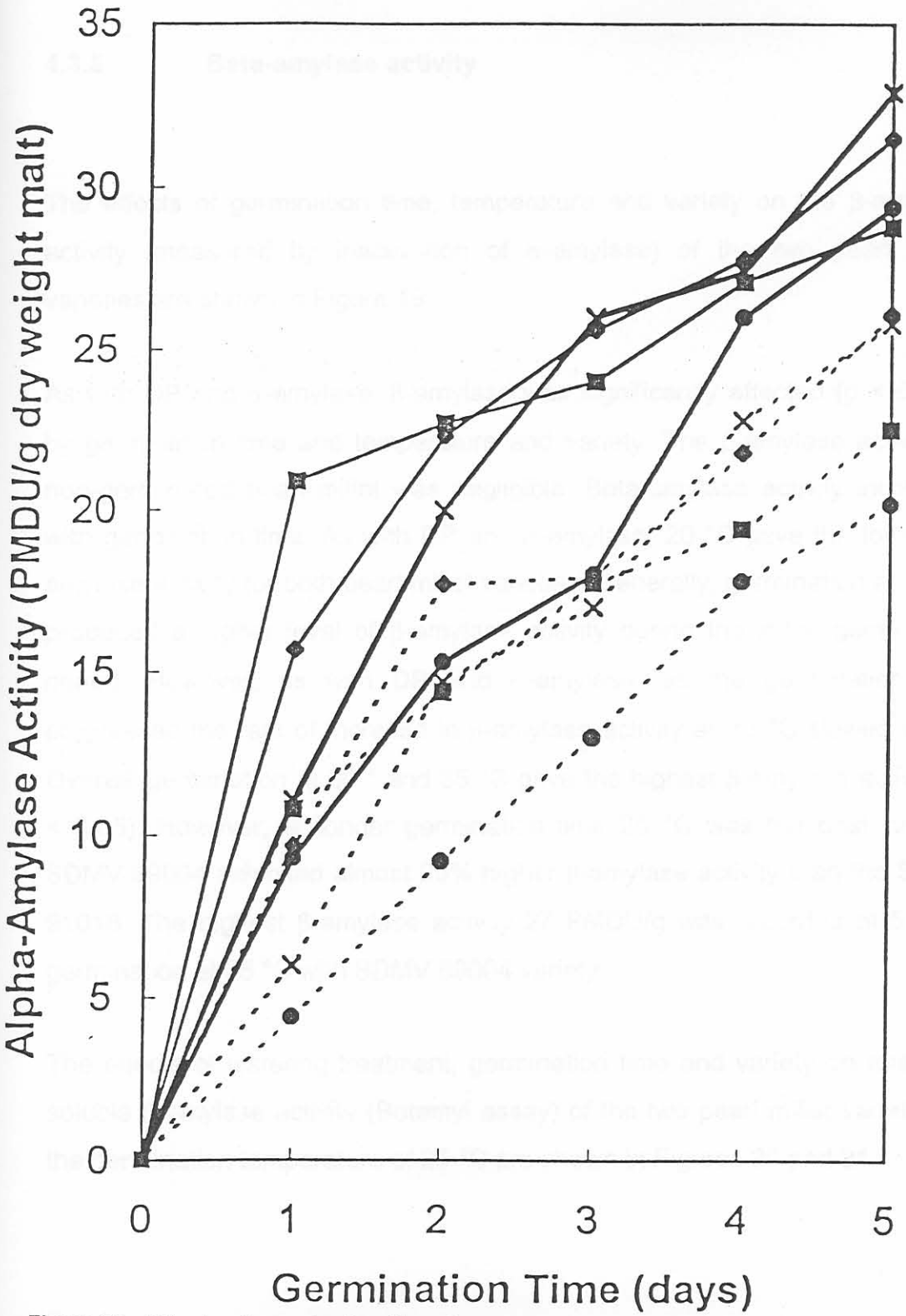

Figure 18.- Effects of germination time, temperature and variety on the α-amylase activity of pearl millet (variety SDMV 89004 (–) and variety SDMV 91018 (--); (•- 20 °C; ×- 25 °C; ♦- 30 °C; ■- 35 °C)

4.3.5 Beta-amylase activity

The effects of germination time, temperature and variety on the β-amylase activity (measured by inactivation of α-amylase) of the two pearl millet varieties are shown in Figure 19.

As with DP and α-amylase, β-amylase was significantly affected ($p < 0.001$) by germination time and temperature and variety. The β-amylase activity of non-germinated pearl millet was negligible. Beta-amylase activity increased with germination time. As with DP and α-amylase, 20 °C gave the lowest β-amylase activity for both pearl millet varieties. Generally, germination at 35 °C produced a higher level of β-amylase activity during the initial germination period. However, as with DP and α-amylase, as the germination time progressed the rate of increase in β-amylase activity at 35 °C slowed down. Overall, germination at 30 ° and 35 °C gave the highest β-amylase activity ($p < 0.05$). However, at longer germination time 25 °C was the best. Variety SDMV 89004 exhibited almost 25% higher β-amylase activity than the SDMV 91018. The highest β-amylase activity 27 PMDU/g was recorded at 5 days germination at 35 °C with SDMV 89004 variety.

The effects of watering treatment, germination time and variety on total and soluble β-amylase activity (Betamyl assay) of the two pearl millet varieties at the germination temperature of 25 °C are shown in Figures 20 and 21.

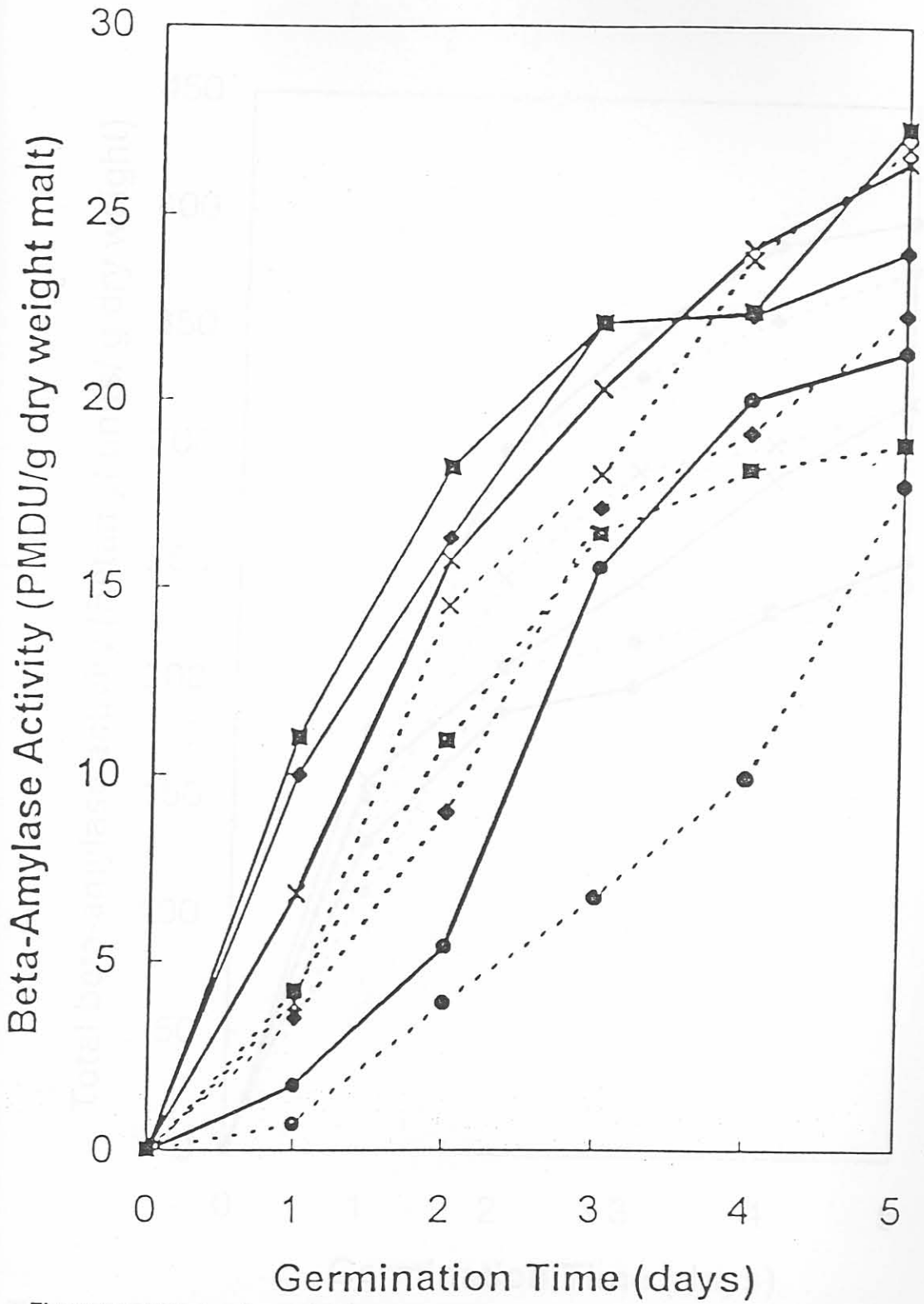

Figure 19.- Effects of germination time, temperature and variety on the β-amylase activity (measured by inactivation of α-amylase) of pearl millet (variety SDMV 89004 (–) and variety SDMV 91018 (--); (•- 20 °C; ×- 25 °C; ♦- 30 °C; ■- 35 °C)

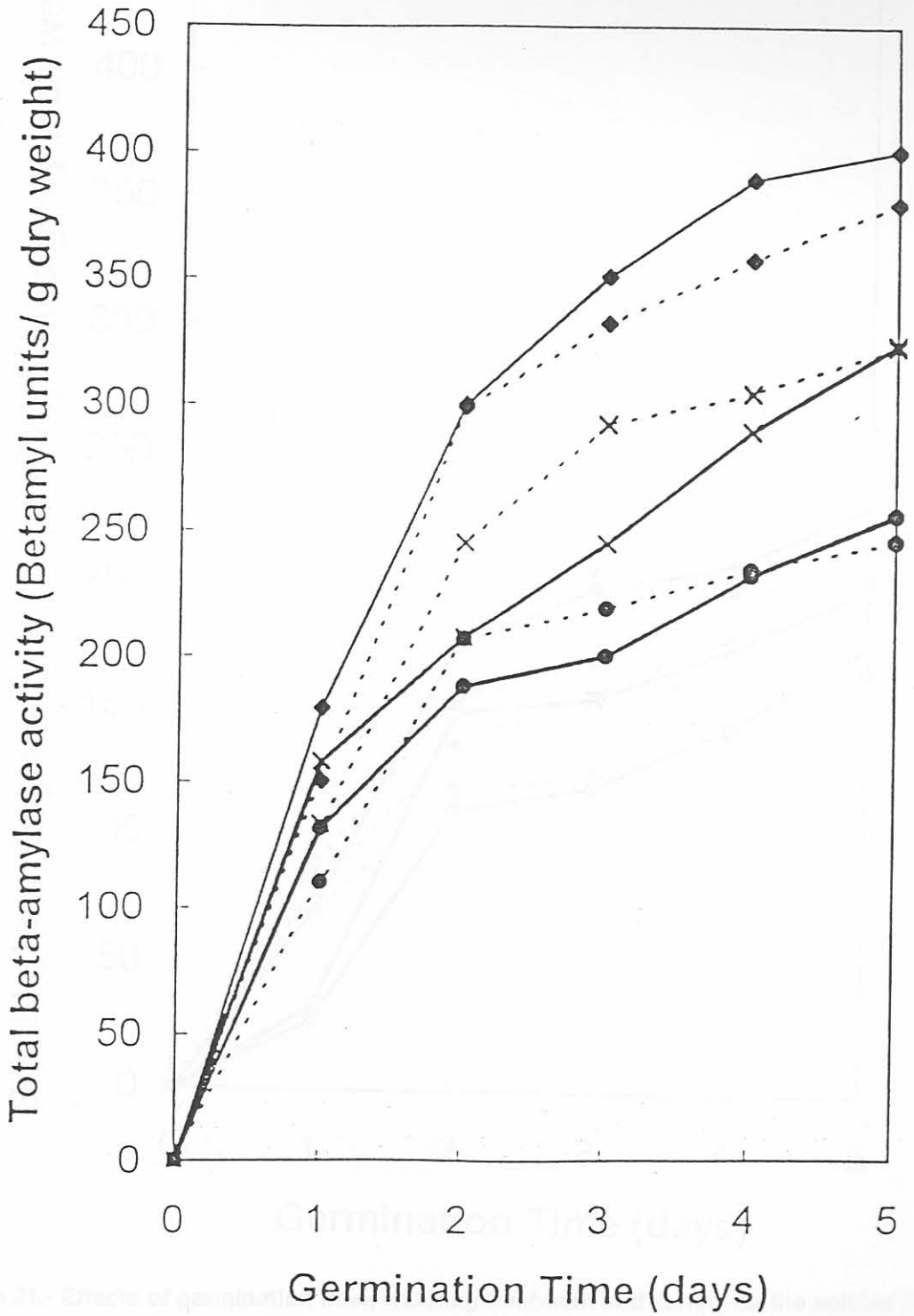

Figure 20.- Effects of germination time, watering treatment and variety on the total β-amylase activity (measured by Betamyl assay) of pearl millet at 25 °C (variety SDMV 89004(–) and variety SDMV 91018 (--) at various watering treatments (•- Low watering; ×- Medium watering; ♦- High watering)

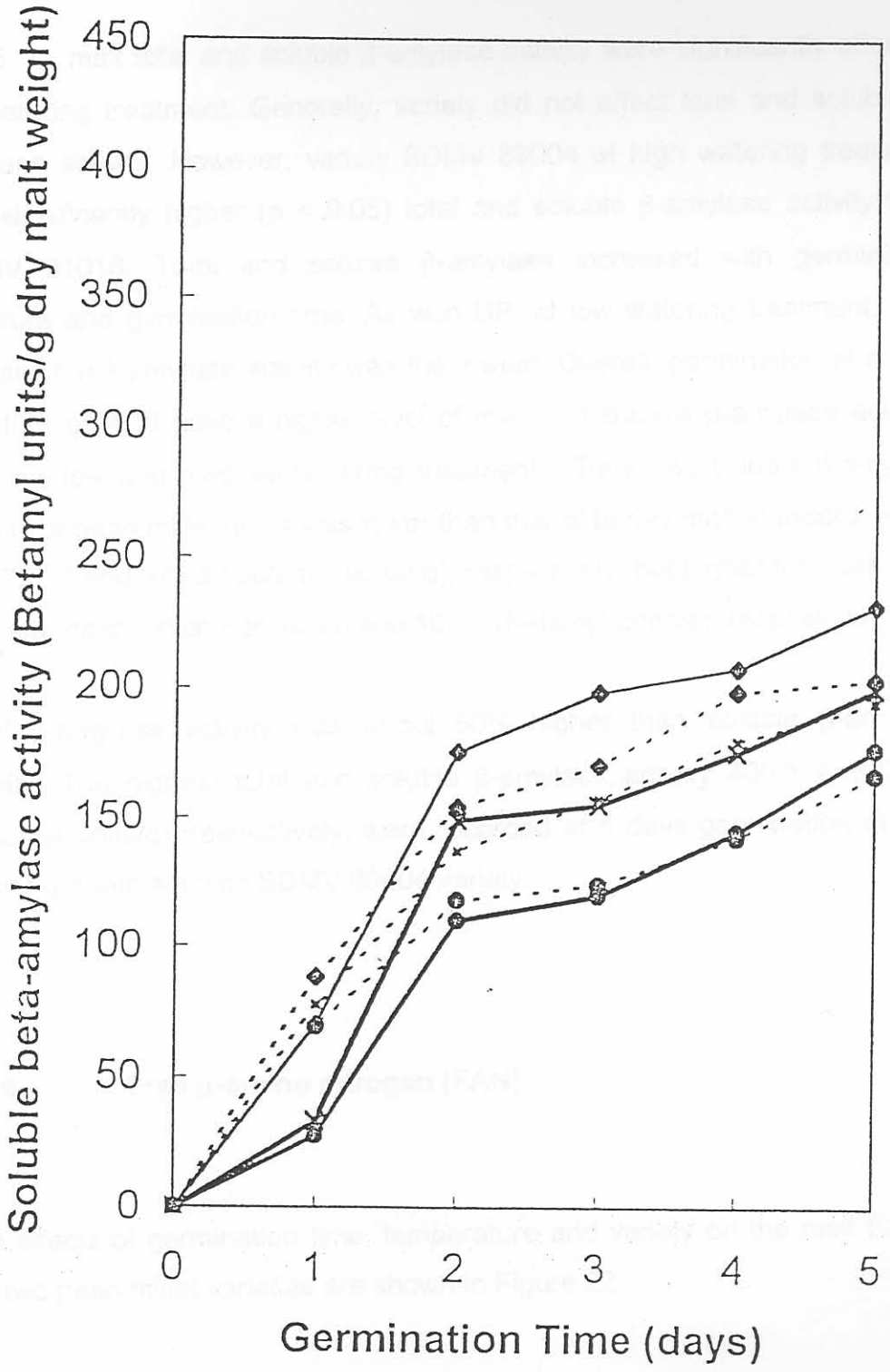

Figure 21.- Effects of germination time, watering treatment and variety on the soluble β-amylase activity (measured by Betamyl assay) of pearl millet at 25 °C (variety SDMV 89004(–) and variety SDMV 91018 (--) at various watering treatments (•- Low watering; ×- Medium watering; ♦- High watering)

At 25 °C, malt total and soluble β-amylase activity were significantly affected by watering treatment. Generally, variety did not affect total and soluble β-amylase activity. However, variety SDMV 89004 at high watering treatment had significantly higher ($p < 0.05$) total and soluble β-amylase activity than SDMV 91018. Total and soluble β-amylase increased with germination moisture and germination time. As with DP, at low watering treatment, total and soluble β-amylase activity was the lowest. Overall, germination at a high moisture content gave a higher level of total and soluble β-amylase activity, than the low and medium watering treatments. Total and soluble β-amylase activity of pearl millet malts was lower than that of barley malt standard, which had 727.7 and 375.5 (Betamyl units/ g), respectively, but higher than sorghum malt standard, which had 203.0 and 108.8 (Betamyl units/g), respectively.

Total β-amylase activity was about 50% higher than soluble β-amylase activity. The highest total and soluble β-amylase activity 400.1 and 232.0 (Betamyl units/g), respectively, were recorded at 5 days germination at high watering treatment with SDMV 89004 variety.

4.3.6 Free α-amino nitrogen (FAN)

The effects of germination time, temperature and variety on the malt FAN of the two pearl millet varieties are shown in Figure 22.

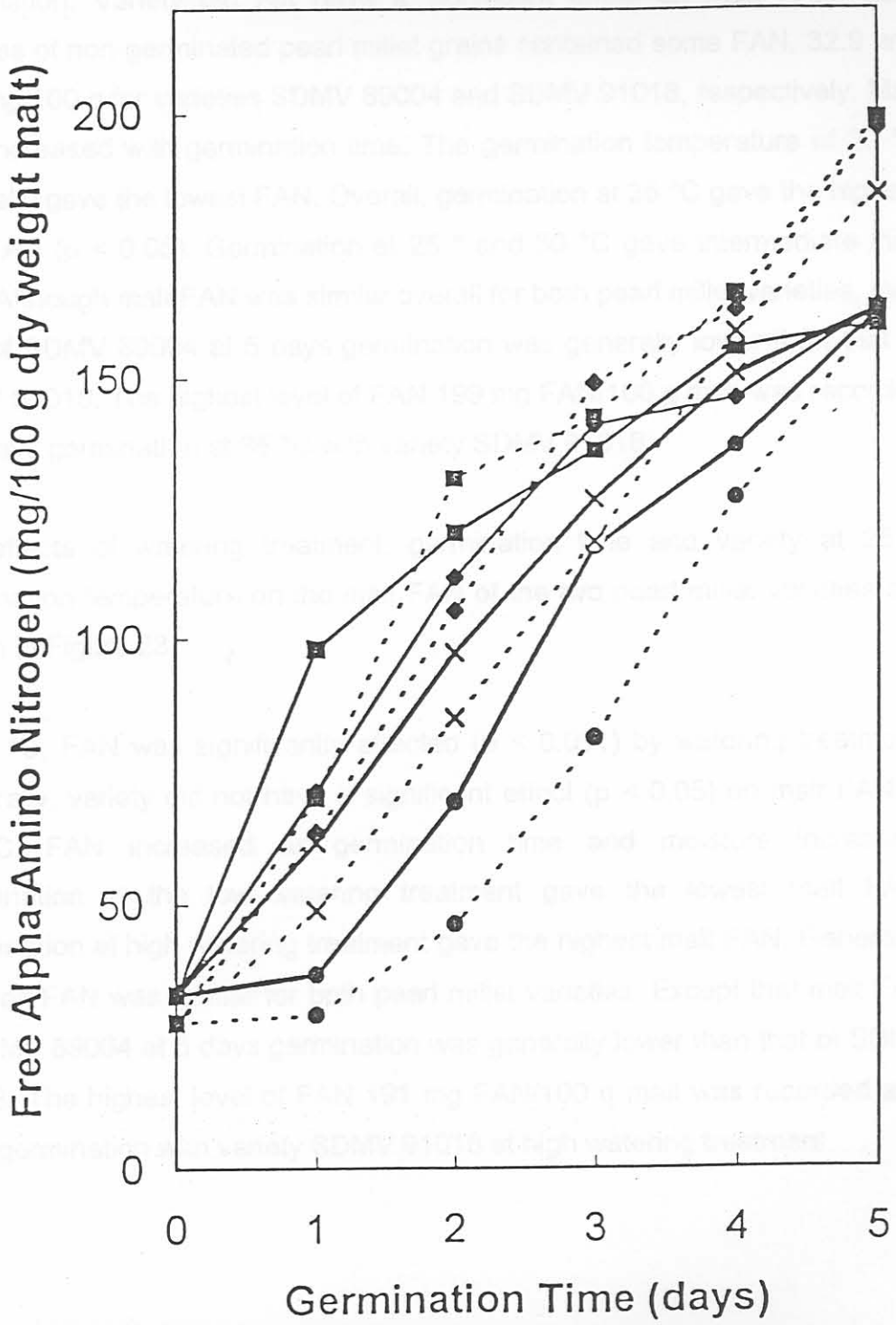

Figure 22.- Effects of germination time, temperature and variety on the malt FAN of pearl millet (variety SDMV 89004 (–) and variety SDMV 91018 (--); (•- 20 °C; ×- 25 °C; ♦- 30 °C; ■- 35 °C)

FAN was significantly affected ($p < 0.001$) by time and temperature of germination. Variety did not have a significant effect on malt FAN. Both varieties of non-germinated pearl millet grains contained some FAN, 32.9 and 27.6 mg/100 g for varieties SDMV 89004 and SDMV 91018, respectively. Malt FAN increased with germination time. The germination temperature of 20 °C generally gave the lowest FAN. Overall, germination at 35 °C gave the highest malt FAN ($p < 0.05$). Germination at 25 ° and 30 °C gave intermediate malt FAN. Although malt FAN was similar overall for both pearl millet varieties, malt FAN of SDMV 89004 at 5 days germination was generally lower than that of SDMV 91018. The highest level of FAN 199 mg FAN/100 g malt was recorded at 5 days germination at 35 °C with variety SDMV 91018.

The effects of watering treatment, germination time and variety at 25°C germination temperature on the malt FAN of the two pearl millet varieties are shown in Figure 23.

At 25 °C, FAN was significantly affected ($p < 0.001$) by watering treatment. Generally, variety did not have a significant effect ($p < 0.05$) on malt FAN at 25 °C. FAN increased as germination time and moisture increased. Germination at the low watering treatment gave the lowest malt FAN. Germination at high watering treatment gave the highest malt FAN. Generally, the malt FAN was similar for both pearl millet varieties. Except that malt FAN of SDMV 89004 at 5 days germination was generally lower than that of SDMV 91018. The highest level of FAN 191 mg FAN/100 g malt was recorded at 5 days germination with variety SDMV 91018 at high watering treatment.

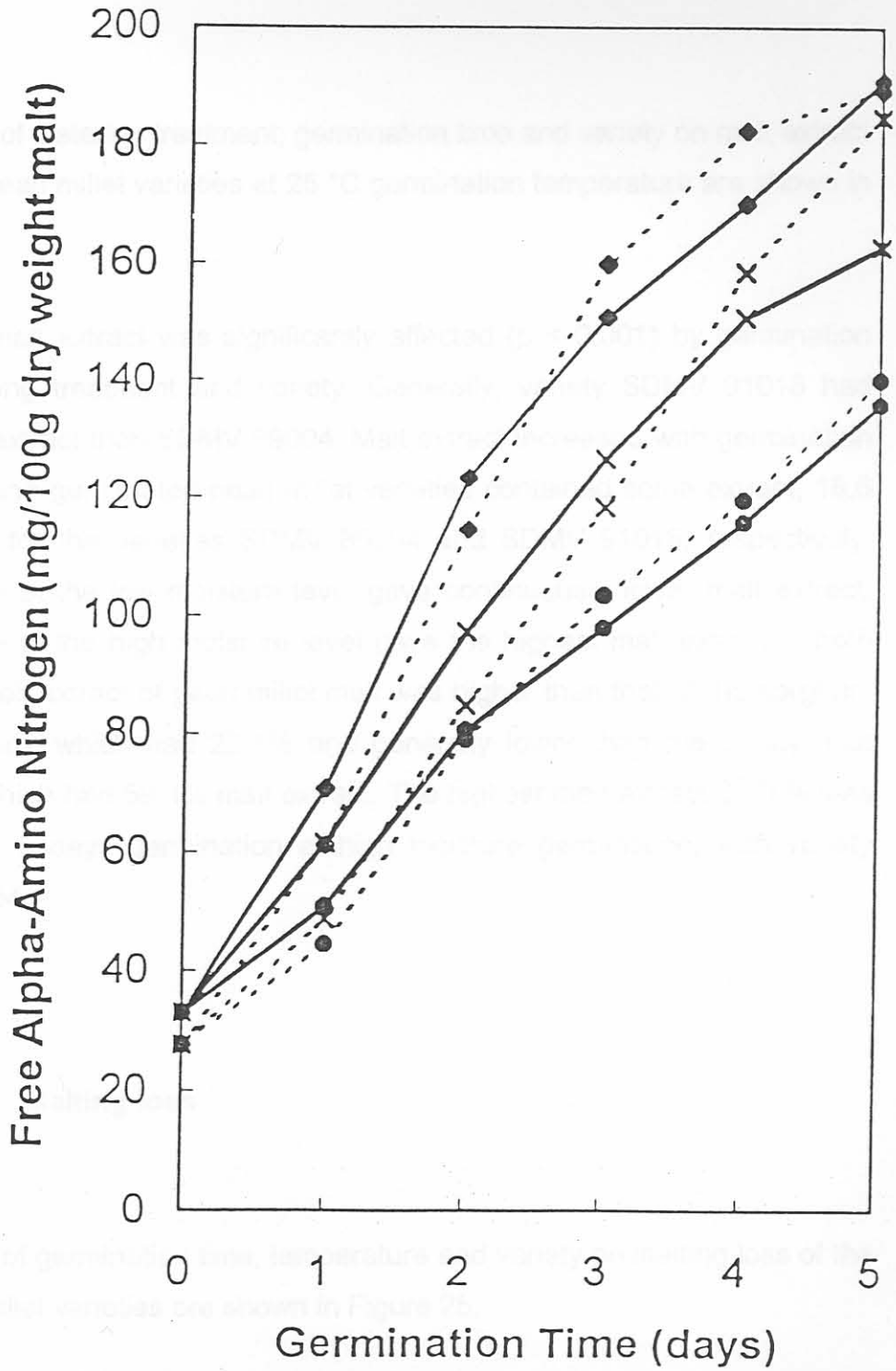

Figure 23.- Effects of germination time, watering treatment and variety on the malt FAN of pearl millet at 25 °C (variety SDMV 89004(–) and variety SDMV 91018 (--) at various watering treatments (•- Low watering; ×- Medium watering; ♦- High watering)

4.3.7 Hot water extract

The effects of watering treatment, germination time and variety on malt extract of the two pearl millet varieties at 25 °C germination temperature are shown in Figure 24.

Hot water malt extract was significantly affected ($p < 0.001$) by germination time, watering treatment and variety. Generally, variety SDMV 91018 had lower malt extract than SDMV 89004. Malt extract increased with germination time. Both non-germinated pearl millet varieties contained some extract, 18.6 and 18.5% for the varieties SDMV 89004 and SDMV 91018, respectively. Germination at the low moisture level gave continuously lower malt extract. Germination at the high moisture level gave the highest malt extract in both varieties. Malt extract of pearl millet malt was higher than that of the sorghum malt standard, which had 22.1% and generally lower than the barley malt standard, which had 59.4% malt extract. The highest malt extract 69.0 % was recorded at 5 days germination at high moisture germination, with variety SDMV 89004.

4.3.8 Malting loss

The effects of germination time, temperature and variety on malting loss of the two pearl millet varieties are shown in Figure 25.