Yield and quality of grain amaranth (*Amaranthus* sp.) in Eastern Austria

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ABSTRACT

The introduction of a new crop requires adapted genotypes as well as optimum crop management practices. This study was conducted to determine the optimum crop density of adapted grain amaranth genotypes in the Pannonian region of Eastern Austria. The genotypes Neuer Typ (*A. hypochondriacus*), Mittlerer Typ (*A. hypochondriacus*) and Amar (*A. cruentus*) were established at plant densities of 8, 17 and 35 plants/m^2 in 2002 and 2003. Average hand-harvested yields ranged from 2200 to 3000 kg/ha without significant genotypic differences. Genotypes differed in thousand seed weight (0.55–1.04 g), time from sowing to harvest (97–130 days), grain water content at harvest (24–38%), microbial infestation of air-dried grain (0.2–118.6 cfu × 10^6/g), germination (29–79%) and grain composition. Grain contents fell within the following ranges: crude protein 15.2–18.6%, crude fat 5.4–8.6%, crude fibre 3.5–4.2%, ash 2.7–3.2%, and carbohydrates 66.7–72.7%. High grain water contents involved stronger microbial infestation and reduced germination. Crop density affected neither grain yield nor grain quality.

Keywords: *Amaranthus*; genotype; plant density; grain yield; water content; thousand seed weight; chemical composition; microbial infestation; germination

Amaranth, still a rarely grown specialty crop in Europe, is a dicotyledonous C4 species that originates from Central and South America. At present, the commercial interest in amaranth grain is restricted to the health food market. Moreover, some consumer attention is due to the colourful history of the exotic crop (Myers 1996). To be economically competitive with other grain crops, amaranth requires respectable grain yields and high quality levels of the grain. These objectives may be achieved partly by optimal crop management and partly by selection and breeding.

The origin rather than the botanical species is the decisive factor for a suitable genotype (Kaul et al. 1996). Breeding targets are high yielding, short, early and uniformly maturing lines with reduced seed shattering and large seeds of high nutritional value (Fitterer et al. 1996, Brenner 2002). Under long photoperiods of the temperate climate, grain amaranth is threatened by late grain maturation and slow plant drydown which give rise to harvest difficulties and grain losses. In humid climates, e.g. in Southwest Germany, amaranth grain is harvested after a growing period of 130 to 150 days, with water content of 25–30% (Aufhammer 2000). High moisture content of plants and grain implies ideal growing conditions for microbes thus debasing grain quality (Krishnan et al. 1994, Aufhammer 2000).

Especially genotypes with dense inflorescences can favour grain mould problems by retaining moisture (Brenner et al. 2000). Bresler et al. (1998) found moisture contents of 13.5% suitable for safe storage at 25°C to prevent fungal growth on amaranth grain. The most important physical quality trait of amaranth seed is thousand seed weight, which

ranges from 0.3 to 1.2 g (Williams and Brenner 1995). Large seeds facilitate mechanical sowing as well as grain processing like milling and popping, but improving seed size by selection is difficult (Brenner et al. 2000). Chemical grain composition varies within the *Amaranthus* species (Bressani 1994, Williams and Brenner 1995, Muchová et al. 2000). Usually, grain contents fall within the following ranges: crude protein 12–19%, fat 5–8%, starch 62–69%, total sugars 2–3%, fibre 4–5% and ash 3–4% (Williams and Brenner 1995).

Recommendations for the optimal crop density differ substantially. Guillen-Portal et al. (1999) established grain amaranth in the wide range of 4–200 plants/m^2 on dry lands of Nebraska and achieved maximum yield at 47 plants/m^2. Henderson et al. (2000) found no yield response to populations between 7 and 27 plants/m^2 in Dakota. Neither did Myers (1996) with seeding rates from 0.28 to 4.40 kg/ha in Columbia. Testing 6–22 plants/m^2, Apaza-Gutierrez et al. (2002) achieved the best yields at 22 plants/m^2 in field studies in Bolivia. At densities from 10–150 plants/m^2 in Southwest Germany, 60–70 plants/m^2 proved to be the optimum density (Aufhammer 2000). Studies might be inconclusive because amaranth is able to compensate for different levels of plant population due to its plastic morphology. Moreover, the optimum density depends on site and variety (Henderson et al. 2000). Little is known about the effect of crop density on quality parameters, but Aufhammer and Kübler (1998) suggested that rising interplant competition in dense stands might result in earlier vegetative drydown of the crops and more synchronous grain maturation thus enhancing biological quality standards. Glowienke and Kuhn (1998) found impacts of plant density on mineral composition of amaranth grain. Likewise density trials on other crops suggest that plant population might affect grain components of amaranth (Andrade and Ferreiro 1996).

The first objective of this study was to evaluate grain yield, physical, chemical and biological quality traits of grain amaranth genotypes adapted to the Pannonian region in Eastern Austria; the second one was to determine the most desirable plant population to maximize yield and optimize quality traits.

MATERIAL AND METHODS

Field experiments were conducted at the Experimental Farm Gross-Enzersdorf (48°12'N, 16°33'E, 153 m above sea level) in Eastern Austria during 2002 and 2003 growing seasons (Table 1). Mean annual precipitation is 546 mm, mean annual temperature is 9.8°C. Figure 1 gives detailed information on weather conditions. The soil type at the experimental site is a chernozem (silty loam to loamy silt, 2.2% humus, pH-value 7.6). In 2002, the experiment design was a randomized complete block in a split plot arrangement with four replications. Genotype was the main plot factor; plant density was the subplot factor. In 2003, all treatments were randomised in blocks with three replications. Plots were 10 m in length and consisted of eight rows spaced 37.5 cm apart. Seed was machine planted and hand-thinned at the eight-leaf stage to achieve final plant stands of 8, 17 and 35 plants/m^2. At harvest, final population densities (Table 2) were determined by counting hand-harvested plants.

Three genotypes adapted to the Pannonian climate were selected for this study. Seeds were obtained from the breeder G. Dobos, ZENO PROJEKTE, Vienna, Austria. Neuer Typ (*A. hypochondriacus*) is a highly branching, early maturing, semidwarf type. Mittlerer Typ (*A. hypochondriacus*), a selec-

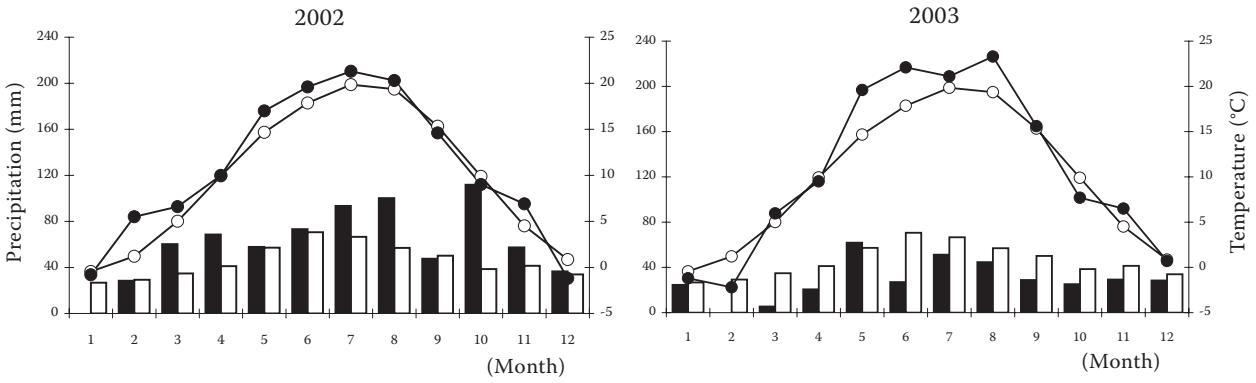

Figure 1. Total monthly precipitation (solid bars), long-term average monthly precipitation (open bars), ● mean monthly temperature, and ○ long-term average monthly temperature during 2002 and 2003 growing seasons

Table 1. Field experiments during 2002 and 2003 growing seasons

	2002	2003
Sowing date	April 29	May 7
Fertilisation	none	none
Soil mineral N (0–90 cm)	March 14: 135 kg N/ha	April 3: 54 kg N/ha May 8: 303 kg N/ha
	Harvest date	
Neuer Typ	August 19	August 7
Mittlerer Typ	September 5	August 25
Amar	September 18	September 1

tion from a cross made by B. Baji (the Institute for Agrobotany, Tapioszele, Hungary), is medium in maturity and tall with little tendency to branch. Amar (*A. cruentus*, Mexican type), a selection from RRC 1041 (Rodale Research Center), is a late maturing, sparsely branching, tall type with dense inflorescences.

A 2-m section of two centre rows (1.5 m^2) was hand-harvested by cutting stems at the soil surface. Harvest date was set in accordance with plant senescence (Table 1). However, genotypes were very different in plant drydown and grain maturation. The late genotype Amar did not dry down under prevailing conditions; the harvest date was therefore set before autumn in order to minimize grain losses due to wind and heavy rainfalls. Dried inflorescences were threshed by a stationary ear thresher. Grain dry matter weights were obtained by drying cleaned grain samples at 100°C for 24 hours.

Grain harvested by a plot combine was used for the determination of seed quality parameters. Seeds were cleaned and air-dried on greenhouse tables to 8–12% moisture content for storage at room temperature. All physical and analytical results are expressed on a dry weight basis. Grain moisture at harvest was obtained by drying a 100 g sample (100°C, 24 hours) immediately after harvest. Seed counts (1000 seeds per plot) were made using the Pfeuffer-Contador seed counter.

Chemical grain composition was analysed in duplicate. Residual water content of stored samples was determined according to the ICC standard No. 110/1. The content of crude protein was calculated by multiplying nitrogen content by 6.25. Nitrogen content of a 500 mg sample of whole seeds was determined by the Leco CN-2000 analyser. Ground seeds (Retsch ZM100 mill, 0.25 mm ring sieve) were used for the determination of all other chemical components. Analysis of fat content was carried out with a 5 g sample using the Soxhlet extraction procedure with petrolether as solvent. The content of crude fibre was determined with 1 g samples using the FibreCap system 2021 according to the AOAC standards. Defatting of samples in polypropylene cups was done with petrolether. Digestion steps were performed using sodium hydroxide, sulphuric acid and hydrochloric acid. Dried cups were incinerated in a muffle furnace (600°C). Ash content was determined by incineration of 2 g samples in a muffle furnace (900°C). Finally, carbohydrates were calculated as the difference to 100%.

Table 2. Desired and established plant populations

Year	Desired populations (plants/m^2)	Established populations at harvest (plants/m^2)			
		mean across all genotypes	Neuer Typ	Mittlerer Typ	Amar
2002	8	7	8	7	6
	17	14	14	14	15
	35	32	33	27	37
2003	8	10	10	10	10
	17	18	20	17	17
	35	36	35	43	29

The number of aerobic mesophilic bacteria was determined using the plate count method (ICC standard No. 125). 40 g samples of grain were blended with sterile NaCl-peptone. Decimal dilutions were prepared. 1 ml of each dilution was pipetted (duplicate) into Petri dishes and mixed with 15 ml of added casein-peptone-glucose-yeast extract agar (cooled to 45 ± 1°C). With solidified agar, Petri dishes were inverted and incubated for 72 ± 2 hours at 30°C. Plate counts were computed from plates within a range of 20 to 300 colonies and expressed as number of colony forming units per gram grain (cfu/g). For statistical analysis, plate counts were transformed into logarithmic data (logarithm to the basis 10).

Germination tests were carried out in Petri dishes exposed to light in climatic test cabinets at the temperature of 20°C. 4 × 50 seeds per plot were put on saturated filter paper for 14 days. The papers were moistened with 0.2% KNO_3 solution instead of water to break seed dormancy as recommended for ornamental *Amaranthus species* by ISTA (1993).

Data were analysed using the SAS version 8. Analyses of variance were performed for the data of each year separately. Multiple comparisons of means were performed using the test of Student-Newman-Keuls at $P = 0.05$. Differences among years were not tested because of different experimental designs. Interrelations between traits were tested by computing the coefficient of determination for linear regressions.

RESULTS AND DISCUSSION

Genotypes showed similar hand-harvested grain yields, but were different for thousand seed weight (Table 3), grain composition (Table 4), grain moisture at harvest, microbial infestation and germination percentage (Table 5). Although genotypes statistically produced similar grain yield, Neuer Typ and Amar tended to produce higher grain yields than Mittlerer Typ. Mittlerer Typ is the tallest genotype and showed a tendency to lodge. In 2002, the semidwarf Neuer Typ produced the highest grain yields. The greatest yields occurred in 2003 with Neuer Typ and Amar; this result may be attributed to the warm growing season. In general, achieved yield levels were similar to hand-harvested yields of *A. cruentus* and *A. hypochondriacus* × *A. hybridus* lines (between 2500 and 3300 kg/ha) in Southwest Germany (Aufhammer and Kübler 1998) and average grain yields of *A. hypochondriacus* and *A. cruentus* (between 2100 and 2700 kg/ha) in the Slovak Republic (Jamriška 1996, 2002). Losses caused by seed shattering were not measured, but would be of further interest especially with respect to mechanical harvesting.

Thousand seed weight (Table 3) was within the range of 0.5–1.0 g, which is similar to seed weights of *A. hypochondriacus* and *A. cruentus* genotypes tested by Jamriška (1996) and Kaul et al. (1996). Thousand seed weight of genotypes Mittlerer Typ and Amar was low, while Neuer Typ produced the highest thousand seed weight. The large seed size enabled the semidwarf Neuer Typ to exhibit high grain yields. In contrast, high yields of Amar seemed to result from a high number of small seeds.

The proximate chemical composition of seeds of the three amaranth genotypes tested is shown in Table 4; they showed significant differences in the content of carbohydrates, crude protein, crude fat and ash, while the content of crude fibre was not affected by genotype. Low crude protein values and the lowest crude fat values of Neuer Typ went along

Table 3. Grain yield (dry matter basis) and thousand seed weight (dry matter basis) of three genotypes

Year	Genotype	Grain yield (hand-harvested) (kg dm/ha)	1000-seed weight (g dm)
2002	Neuer Typ	2700 a*	0.96 a
	Mittlerer Typ	2202 a	0.63 b
	Amar	2391 a	0.67 b
2003	Neuer Typ	2955 a	1.04 a
	Mittlerer Typ	2479 a	0.55 b
	Amar	3006 a	0.67 b

*numbers with different letters are significantly different ($P = 0.05$)

Table 4. Proximate grain composition (in %) of three genotypes

Year	Genotype	Carbohydrates	Crude protein	Crude fat	Crude fibre	Ash
2002	Neuer Typ	71.27 a*	15.76 b	6.08 c	3.77 a	3.12 ab
	Mittlerer Typ	66.71 b	18.55 a	7.29 b	4.22 a	3.23 a
	Amar	68.01 b	16.39 b	8.60 a	3.96 a	3.04 b
2003	Neuer Typ	72.72 a	15.22 c	5.40 c	3.94 a	2.73 b
	Mittlerer Typ	68.71 c	17.55 a	6.64 b	4.10 a	2.99 a
	Amar	70.61 b	15.79 b	7.35 a	3.54 a	2.71 b

*numbers with different letters are significantly different (P = 0.05)

with the highest contents of carbohydrates. Mittlerer Typ was characterised by clearly the highest contents of crude protein, highest contents of minerals, medium contents of crude fat, and lowest contents of carbohydrates. Amar showed the highest crude fat values, lowest mineral contents and low protein contents. The protein content of Amar was slightly higher than that of Neuer Typ, but this difference was significant only in 2003. Among the tested genotypes, increasing seed weights went along with rising contents of carbohydrates and decreasing contents of crude protein. The grain of amaranth is covered by the seed coat, a poorly developed endosperm, an annular embryo, and a central zone, the starchy perisperm. It is known that the germ fraction contributes significant amounts of protein to the whole seed (Bressani 1994). This is the reason why the proportion of the germ with respect to the whole grain is a factor that can influence seed composition. Increasing seed size predominantly affects the starchy portion of the perisperm as opposed to the germ. Hence it can have an effect of reducing seed protein (Brenner et al. 2000). The protein content of amaranth seems to be negatively correlated with yield like it is in other grain crops (Feil 1998). Therefore, high protein contents of Mittlerer Typ may account for the lowest yields.

Under prevailing growing conditions, the earliest genotype Neuer Typ reached maturity after a vegetation period of 14–16 weeks (Table 5). Mittlerer Typ could be harvested two weeks later, Amar three to four weeks later. Grain of all genotypes needed drying for storage due to high grain moisture at harvest. Neuer Typ and Mittlerer Typ showed similar grain water contents, while grain water contents of the late maturing Amar were significantly higher.

Aerobic plate counts are useful for indicating the overall microbiological quality (Table 5). The threshold below which results are considered satisfactory is defined as "threshold m" (International Commission on Microbiological Specifications for Foods, 1986). According to the guidelines

Table 5. Days from sowing to harvest, grain moisture, microbial infestation, and germination of three genotypes

Year	Genotype	Days from sowing to harvest	Grain moisture at harvest (%)	Colony forming units (cfu × 10^6/g)**	Germination (%)
2002	Neuer Typ	113	23.9 b*	10.9 b	62 a
	Mittlerer Typ	130	27.6 b	5.7 b	29 b
	Amar	143	32.8 a	118.6 a	***
2003	Neuer Typ	97	27.2 b	2.0 b	72 a
	Mittlerer Typ	112	28.4 b	0.2 c	79 a
	Amar	119	38.4 a	20.1 a	54 b

*numbers with different letters are significantly different (P = 0.05); **statistical analysis of log-transformed data; ***not available, hardly any germination observed

of the Deutsche Gesellschaft für Hygiene und Mikrobiologie, the "threshold m" for ground cereal products for raw consumption amounts to 10^6 colony forming units (cfu)/g (Baumgart and Becker 2004). In 2002, studied samples of all genotypes exceeded the satisfactory threshold, samples of Amar even more than 100-fold. In 2003, the bulk contamination rates of Mittlerer Typ and Neuer Typ were satisfactory whereas Amar showed the highest contamination rates beyond the acceptable threshold again. The markedly reduced contamination values in 2003 can be attributed to high temperatures and low precipitation. As expected, the linear regressions between moisture content and microbial contamination, the latter expressed as log (cfu), indicate that high moisture content of grain at harvest entailed ideal growing conditions for microbes (Figure 2). The observed differences among genotypes might also be related to different environmental conditions during maturation or different harvest dates, respectively. Additionally, differences in inflorescence architecture might have contributed to different microclimatic conditions within inflorescences. Hence the very compact, dense inflorescences of the late Amar could have increased the hygienic problems of that genotype. Furthermore, post-harvest handling like storage and drying procedures may have influenced the contamination progress. However, the variation in grain moisture at harvest accounted for more than 50% of the variability in microbial plate counts.

Germination percentages were generally low (Table 5). In 2002, the germination of seeds of Amar was not accomplished due to very low germination percentages of random samples (data not shown). Likewise, Amar showed the lowest germination values in 2003. Increasing microbial contamination impaired germination substantially. This effect was traceable only in 2002 (Figure 3), not in 2003 (data not shown). To obtain high germination rates, hand-harvested, carefully dried grain sources are required.

Substantially different plant densities were achieved by hand-thinning. Consequently, differences in plant morphology were obvious. Dense stands resulted in less branching plants with thinner stems and shorter flower heads (data not shown). However, no significant impacts on evaluated yield and quality traits could be noted. Similar grain yields of the tested density range correspond to the findings of other authors (Myers 1996, Henderson et al. 2000). In contrast to other findings (Andrade and Ferreiro 1996, Glowienke and Kuhn 1998), the proximate analysis of tested grain did not reveal any plant density effects. A tendency was observed that increasing plant densities implicated earlier vegetative drydown and slightly falling grain moisture at harvest (data not shown). Aufhammer et al. (1999) suggested that rising interplant competition going along with shorter heads and more synchronous grain maturation might influence microbial grain quality. Yet, tested densities did not have any impact on microbial infestation.

In conclusion, significant differences among the tested genotypes were noted for seed weight, time to maturity, grain composition and microbial quality. The semidwarf Neuer Typ that is characterised by satisfying yield, early grain maturation and large seed size suited best for growing conditions in Austria. The tallest genotype Mittlerer Typ showed high protein contents, but tended to produce lowest yields. Amar produced satisfying yields but was characterised by late grain maturation and scarce plant drydown. Although different crop densities affected plant morphology, expected impacts on yield and grain quality traits were not identified under prevailing conditions.

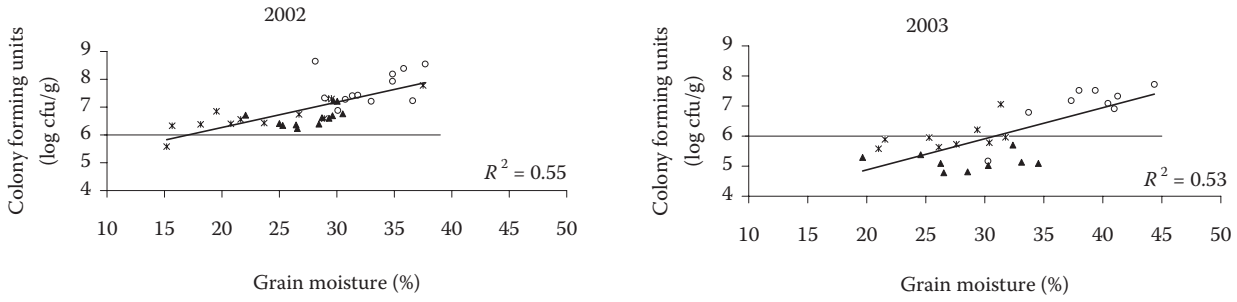

Figure 2. Linear relationship between grain moisture and the logarithm of microbial infestation (∗ Neuer Typ, ▲ Mittlerer Typ, ○ Amar; — satisfactory "threshold m")

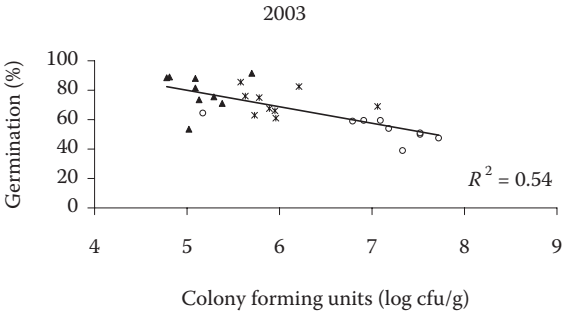

Figure 3. Linear relationship between the logarithm of microbial infestation and germination in 2003 (∗ Neuer Typ, ▲ Mittlerer Typ, ○ Amar)

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