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In vivo haploid induction in maize

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Abstract Two haploid-inducing lines, MHI and M741H, were used for the production of maternal haploids. Haploids were obtained from all maternal genotypes involved in the experiment, including dent, flint and flint×dent maize. The maternal genotype had a significant influence on the frequency of haploids obtained. The frequency ranged from 2.7% to 8.0%. For chromosome-doubling seedlings were treated with colchicine solution, and 49.4% of the haploid plants produced fertile pollen, 39.0% could be selfed and 27.3% produced seeds after selfing. Synthetic populations, improved by haploid sib recurrent selection, were tested in a field trial. The results show that the utilization of maternal haploid plants has great potential for maize breeding and maize genetics.

Keywords In vivo haploids · Haploid-inducing lines · Chromosome doubling · Haploid sib recurrent selection

Introduction

The development of homozygous lines is an important part of maize breeding. Traditionally, homozygous lines are obtained by 5–6 selfings of heterozygous material, a time-consuming and expensive process.

Here, maternal haploids based on the availability of special genotypes related to the line Stock 6 (Coe 1959) are used in obtaining doubled-haploid lines (DH lines). For identifying haploids, a system of dominant anthocyanin marker genes is used, described and widely applied by Nanda and Chase (1966) and Chase (1969). In this system, the *R1-nj* gene is used to distinguish haploid and diploid plants. The expression of this gene provides an anthocyan pigmentation of the embryo and the endosperm. Kernels with a pigmented endosperm and a non-pigmented embryo were selected as haploids. The procedure for the production of maternal haploids allows obtaining haploids from different genotypes on a large scale (Tyrnov and Zavalishina 1984; Zabirova et al. 1996; Deimling et al. 1997; Chalyk 1999).

Since DH lines are of interest, several methods of chromosome doubling in haploid maize plants have been developed (Zabirova et al. 1996; Deimling et al. 1997).

Doubled-haploid lines have been used for recurrent selection in winter barley (Foroughi-Wehr and Wenzel 1993). Several schemes were developed and tested. Recurrent selection altering with haploid steps was very effective for combining agronomic traits. Hence, it was suggested to use haploid plants also for recurrent selection in maize (Chalyk and Rotarenco 1999).

The mechanism of haploid-inducing capacity in maize has not been understood untill now. It was assumed that in lines inducing haploids two sperms are developed with a different speed (Bylich and Chalyk 1996). As a result one of the sperms develops to a state ready for fertilization, but the other one does not. The existence of only a single normal sperm in a pollen grain would be the reason for a broken doubled fertilization and the development of kernels with a haploid embryo (Enaleeva et al. 1996), but Mahendru and Sarkar (2000) could not find any difference between the two sperms in a line inducing haploids.

Since utilization of the technology for inducing maize haploids in vivo is very promising, our intention was to check it's efficiency in obtaining haploids from genotypes that are widely used in maize breeding in Central Europe.

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Materials and methods

Haploid-inducing lines

Two haploid-inducing lines were tested, MHI (Moldovian Haploid Inducer), created at the Institute of Genetics, Moldova (Chalyk 1999), and M741H, provided by the Maize Genetics Cooperation-Stock Center, USDA/ARS. To compare the effectiveness of the two inducers five maternal genotypes were pollinated with pollen of MHI and M741H.

The isolation of haploids was performed by means of the anthocyanin marker gene, *R1-nj* (Chase 1969). The final identification of haploid plants was done visually in the field and greenhouse, and by counting chromosome numbers.

Line MHI was grown under two different conditions, field and greenhouse, to evaluate the environmental effect on the frequency of haploids obtained. In the greenhouse temperatures, light and water supply were managed optimally for maize growth. In the field, the temperatures during tasseling, flowering and seed formation, were much lower than optimum. Twelve maternal genotypes were pollinated with pollen of the MHI line that grew in the two different environments.

Maternal genotypes

The effect of the maternal genotype on the frequency of haploid induction was evaluated. Twenty different genotypes were included in the experiment, listed in Table 1. These were synthetic populations, single crosses and three-way crosses including five flint genotypes, 11 dent genotypes and four flint×dent hybrids. They were pollinated with pollen of the MHI line. During the isolation of haploids, *R1-nj* gene expression on the kernels' embryo and endosperm of each ear was evaluated.

Chromosome doubling

Two methods were used for chromosome doubling in haploid plants. One has been developed by Deimling et al. (1997), method I, and the other by Zabirova et al. (1996), method II. According to method I, haploid seedlings were treated in the stage when their coleoptile was at least 1 cm long. The seeds were germinated at 26°C during 4 days. Then a small tip of the coleoptile was cut and the seedlings were placed into 0.06% colchicine solution with 0.5% dimethyl sulfoxide (DMSO) for 12 h. The treatment was carried out at room temperature. Thereafter the seedlings were washed in tap water for 20 min and then planted in the field.

According to method II, haploid seedlings were grown untill the 3–4 leaf stage. Then an injection of a 0.125% colchicine solution with 0.5% DMSO was made into a point 3-5 mm above the apex. After the treatment, the haploids were planted both in the field and the greenhouse.

Haploids obtained from the hybrid MK01y×A619 were used for the evaluation of different methods of colchicine treatment. The effect of the treatment was considered to be successful if at least several anthers with fertile pollen appeared on the tassel of a haploid plant. The fertility of pollen was evaluated visually as suggested by Deimling et al. (1997).

Haploid sib recurrent selection

The procedure of haploid sib recurrent selection (HSRS) was previously described by Chalyk and Rotarenco (1999). Each cycle of selection consists of two steps. The first step is to obtain maternal haploids from a synthetic population. The second step is growing haploid plants, the selection of haploid plants with desired traits and pollination with a mixture of pollen collected from diploid plants of the same synthetic population at the same cycle of selection. Seeds harvested from haploids are used for carrying out the next cycle of recurrent selection. For the evaluation of its efficiency two synthetic populations, SP and SA, and the results of different cycles of HSRS were tested in the field. Untill the year 2000 two cycles of selection were completed in synthetic SP and three cycles in synthetic SA. In the field trial a randomized block design with four replications of two rows was used, plot size was 10 m^2, plant density 6 per m^2. Plant characteristics were measured after flowering, when plants stopped their growth. Ears were harvested by hand from each plant separately. After drying they were measured, shelled and the productivity of each plant was evaluated. The maturity of seeds was calculated as the difference of the weight before and after drying.

Statistical analyses

Standard algorithms were applied for statistical analyses. Evaluation of the efficiency of the haploid sib recurrent selection was done on the basis of algorithms provided by Hallauer and Miranda (1988).

Results

Frequency of haploids obtained

The frequency of haploids induced was compared for the two paternal lines, MHI and M741H. Five different maternal genotypes produced on average 4.4% kernels with haploid embryos after pollination with pollen of the MHI line. The use of the M741H line resulted in the production of 2.2% of kernels with a haploid embryo (Table 1).

The second factor investigated was the growth condition of haploid-inducing lines. The MHI line was planted in two different environments, in the greenhouse and in the field. The two different conditions tested had no effect on the frequency of haploids. When the MHI line was planted in the field, it induced 5.4% of haploids out of a total of 14,471 seeds, and when it was planted in the greenhouse, the frequency of haploids was 5.1% out of 8,331 seeds.

The third factor tested was the maternal genotype. It had a significant effect on the frequency of haploids obtained (Table 1). Synthetic populations, single crosses and three-way crosses were used as maternal material. The frequency of haploids induced in different maternal genotypes ranged from 2.7% in Hybrid 5 to 8.0% in LBP1446 x LBP 8006. The average rates of haploids produced from dent and flint×dent genotypes were about the same (5.3% and 4.8%, respectively). For the example of Pop 33/00 the possibility to obtain haploids from flint maize in a sufficient rate (5.7%) is demonstrated.

R1-nj gene expression and embryo-missing seeds

In Table 2 the average score of expression of the anthocyanin marker gene, *R1-nj*, evaluated during the isolation of haploids in embryo and endosperm is presented for the dent, flint and flint×dent genotypes. Regarding all the ears of the three groups, the anthocyan pigmentation was between scores 3 and 4 on a scale from 5 for perfect pigmentation to 1 for no pigmentation. The pigmentation

Table 1 Frequency of haploids induced by two lines, MHI and M741H, and the effect of the maternal genotype on the production of haploids

Genotype	Origin	Seeds		
		Total	Haploid, %	Embryo-less, %
Effect of haploid-inducing line[a]				
MHI	Moldova	4,251	4.4±0.3	2.0±0.2
M741H	USA	1,712	2.2±0.4	0.6±0.2
Effect of maternal genotype[b]				
Pop 33/00	Europ. Flint	859	5.7±0.8	0.9±0.3
LBP9047×Pop 31/99	Europ. Flint	1,853	3.0±0.4	1.0±0.2
(UPJ 125×W 9040)×LBP369	Europ. Flint	2,835	3.5±0.3	2.7±0.3
Pop 30/99	Europ. Flint	1,457	4.2±0.5	1.6±0.3
Flint total/average		7,004	3.6±0.2	1.8±0.2
LBP 1446×LBP 8006	Dent	557	8.0±1.2	5.6±1.0
Pop 32/99 LBP	Dent	2,150	6.2±0.5	2.2±0.3
Pop. 9049 LBP	Dent	1,832	4.0±0.5	3.0±0.4
LBP8001×LBP8003	Dent	2,049	5.8±0.5	1.3±0.2
Synthetic 2000-2 LBP	Dent	2,854	6.8±0.5	2.2±0.3
Synthetic 2000-3 LBP	Dent	1,741	5.6±0.6	2.1±0.3
LBP8999×LBP9039	Dent	1,909	2.9±0.4	1.9±0.3
LBP1446×LBP9039	Dent	2,704	7.6±0.5	3.3±0.3
Hybrid 2	Dent	2,244	6.1±0.5	2.3±0.3
Hybrid 5	Dent	2,606	2.7±0.3	2.8±0.3
Hybrid 6	Dent	1,527	3.0±0.4	1.1±0.3
Dent total/average		22,173	5.3±0.2	2.4±0.1
Hybrid 1	Flint x dent	3,092	3.5±0.5	2.0±0.2
Hybrid 3	Flint x dent	1,734	5.7±0.6	3.0±0.4
Hybrid 4	Flint x dent	1,686	7.1±0.7	2.5±0.4
Pop 29/98×LBP 8014	Flint x dent	1,649	3.7±0.5	2.3±0.4
LBP 9040×LPB 8014	Flint x dent	1,466	3.5±0.3	2.9±0.4
Flint×dent total/average		9,627	4.4±0.2	2.4±0.2
Over all maternal genotypes		38,804	4.8±0.1	2.3±0.1

[a] Five maternal genotypes were included in the experiment
[b] The MHI line was applied for the induction of haploids

Table 2 *R1-nj* gene expression in flint, dent and flint×dent seeds

Genotypes	Number of ears	Average rating of pigmentation[a]	
		Endosperm	Embryo
Europ. flint	89	3.5	4.3
Dent	156	3.7	3.8
Flint×dent	71	3.6	3.6

[a] The following score was used:
5 – excellent pigmentation
4 – good pigmentation
3 – poor pigmentation
2 – bad pigmentation
1 – no pigmentation

of the embryos in the flint genotypes was even better than in the dent and flint×dent genotypes. The average score of pigmentation in flint maize was 4.3, in dent it was 3.7.

After pollination with haploid-inducing lines, some seeds of the maternal genotypes developed without an embryo (Table 1). Considering all maternal genotypes examined, the rate was 2.3%.

Obtaining doubled-haploid lines

One of the main advantages of using haploid plants in maize breeding is the acceleration of the production of new homozygous lines. They can be obtained after chromosome doubling and the selfing of haploids. Chromosome doubling is usually done by colchicine treatment. In Table 3 the effectiveness of the two methods applying colchicine is given.

Method I was only tested in the field. In spite of unfavorable conditions in the season 2000, it produced good results; 49.4% of the haploids had fertile pollen, 39.0% were selfed and 27.3% out of all haploids produced seeds after selfing. Thus utilization of method I allowed the production of DH-lines from about each third haploid plant.

Method II was tested in two environments: in the greenhouse and in the field. In the greenhouse, it resulted in 42.4% of the haploids having pollen and 30.5% of all haploid plants could be selfed. The rest of the fertile haploids, 11.9%, showed a delayed shedding of silks and selfing was impossible. In the field, 16.1% haploids were male-fertile. We selfed 11.3% of all haploids and afterwards 8.1% of all haploids produced seeds. This was about three-times less compared to the results obtained in the greenhouse. We suppose that the reduction of the effectiveness of chromosome doubling in the field is the

Table 3 Chromosome doubling in haploid plants under field and greenhouse conditions

Parameter	Field						Greenhouse	
	Control		Method I		Method II		Method II	
	Number	%	Number	%	Number	%	Number	%
Haploids planted	140	100	106	100	140	100	65	100
Haploids alive	131	93.6	77[a]	72.6	124	88.6	59	90.8
Haploids fertile	3	2.3	38	49.4	20	16.1	25	42.4
Haploids selfed	1	0.8	30	39.0	14	11.3	18	30.5
Haploids produced seeds	0	0	21	27.3	10	8.1	18	30.5

[a] The high rate of haploids that died was the result of a fungal attack

Table 4 Observed mean ear traits for each of the cycles of selection and response to selection in SP and SA synthetic populations

Synthetic population	Traits	Cycle of selection				Coefficient of regression[a]		Gain, %	
		C0	C1	C2	C3	b_0	b_1	Per year	Per cycle
SP	Yield, g/plant	134.7	144.5	148.7*	–	135.6	7.00±1.62	2.6	5.2
	Ear length, cm	15.8	16.3*	16.9***	–	15.78	0.55±0.02[b]	1.6	3.3
	Ear diameter, cm	4.55	4.53	4.78***	–	4.53	0.10±0.11	1.2	2.5
	Kernel rows, no.	15.7	15.7	17.1***	–	15.47	0.70±0.40	2.2	4.5
	Kernels per row, no.	29.3	29.7	31.2**	–	29.12	0.95±0.32	1.6	3.2
	Seed set, %	83.7	82.6	83.9	–	83.3	0.10±0.69	0.05	0.1
	Weight of 1,000 kernels, g	256.1	259.9	254.1	–	257.7	–1.00±2.77	–0.2	–0.4
	Seed humidity, %	43.5	44.8	45.4*	–	43.62	0.95±0.20	1.1	2.2
	Plant height, cm	244.3	260.5***	266.3***	–	246.05	10.99±2.98	2.25	4.50
	Ear height, cm	94.8	104.3***	102.1***	–	96.74	3.64±3.40	1.92	3.84
	Leaf length, cm	75.2	76.7*	78.9***	–	75.08	1.84±0.21	1.22	2.44
SA	Yield, g/plant	106.4	129.6***	122.8**	118.5*	114.9	2.95±4.92	1.9	3.8
	Ear length, cm	15.7	16.4**	16.5***	16.4**	15.92	0.22±0.13	0.7	1.4
	Ear diameter, cm	4.10	4.23**	4.18*	4.23**	4.13	0.03±0.02	0.5	1.0
	Kernel rows, no.	13.8	14.5**	14.6**	14.9***	13.94	0.34±0.08	1.3	2.7
	Kernels per row, no.	28.9	31.3***	31.8***	29.6	30.01	0.26±0.73	0.4	0.9
	Seed set, %	82.4	83.0	82.7	82.0	82.75	–0.15±0.21	–0.05	–0.1
	Weight of 1,000 kernels, g	267.1	251.0**	251.4**	258.4	260.83	–2.57±3.72	–0.5	–1.1
	Seed humidity, %	39.1	40.7	40.9	43.0***	39.14	1.19±0.25[b]	1.6	3.3
	Plant height, cm	223.3	236.5***	244.4***	257.3***	223.90	10.99±0.72[b]	2.54	5.07
	Ear height, cm	69.5	83.8***	84.4***	87.5***	73.15	5.44±2.12	4.30	8.59
	Leaf length, cm	71.9	73.9**	74.4**	76.2***	72.04	1.36±0.19[b]	1.01	2.02

[a] b_0 – is an estimate of the C0 mean; b_1 is an estimate of the average rate of response per cycle; *, **, ***, significant difference from C0 at the 0.05, 0.01 and 0.001 probability levels, respectively
[b] Significant response at the 0.05 probability level

result of unusual low temperatures in the season 2000 regarding the requirements of maize. Control haploid plants were mostly male sterile. Just three plants out of 140 investigated in the field produced fertile pollen. It was possible to self one of them but it did not produce seeds.

Efficiency of selection in haploid plants

Generally, haploid and doubled-haploid plants can be used for the detection and selection of genotypes that contain favorable genes (Foroughi-Wehr and Wenzel 1993). Therefore, a study about the utilization of haploid plants in recurrent selection for the improvement of two synthetic populations, SP and SA, has been launched by Chalyk and Rotarenco (1999). The trait selected at the haploid level was ear size.

Testing of synthetic populations in a field trial showed that the selection of favorable genotypes at the level of haploid plants was effective (Table 4). The grain yield in the synthetic SP was increased from 134.7 g/plant in the initial synthetic to 148.7 g/plant in the synthetic of the second cycle of selection. This means that after just two cycles of selection the average productivity of a plant was increased by 14.0 g. This is a gain of 5.2% per cycle of selection or of 2.6% per year. Simultaneously, traits like ear length, ear diameter, the number of rows per ear and the number of kernels per row were improved. The selection of big ears at the level of haploid plants resulted in an increase of ear size of diploid plants and their grain productivity. It is important to note that

by the selection of ears the grain moisture was also increased. In the initial synthetic, the grain moisture content was 43.5%, and in the synthetic of the second cycle of selection, it was 45.4%. It is possible that, by increasing the grain productivity plants of later maturity were selected.

HSRS in synthetic population SA increased the grain productivity as well. This improvement was not the same in different cycles of selection. In the synthetic of the first cycle of selection, the grain productivity was improved greatly compared to the initial synthetic. The grain yield of the initial synthetic was 106.4 g/plant. After the first cycle of selection, the yield was 129.6 g/plant, a gain in productivity of 23.2 g per plant after just one cycle of selection. After the following cycles of selection, C2 and C3, there was no further increase; the grain yield decreased somewhat but not significantly to 122.8 g/plant and 118.5 g/plant respectively.

The moisture of seeds was raised in the synthetic SA following the increase of the grain yield. In the initial synthetic SA, the moisture of seeds at harvesting was 39.1%, and after the first, second and third cycles of selection was 40.7%, 40.9% and 43.0%.

Furthermore, the selection of ear size in haploid plants resulted in an increased size of diploid plants (Table 5). In the synthetic SP, the plant height increased from 244.3 cm in the initial synthetic to 266.3 cm in the second cycle of selection. Ear height and leaf length were increased as well. The same changes were found in plants of the synthetic SA. After three cycles of selection, the plant height was increased from 223.3 cm for the initial synthetic to 257.3 cm in the population of the second cycle of selection. Ear height increased from 71.9 cm to 76.2 cm. The coefficient of regression in the synthetic SA was significant for plant height and leaf length.

Discussion

The line MHI had a haploid-inducing capacity rate twice as high as line M741H. We suppose this difference is genetically determined and further breeding can be carried out to develop new lines with increased haploid-inducing capacity. Presently both inducers, MHI and M741H, offer the possibility to obtain maternal haploids on a large scale from different genotypes.

Difficulties to isolate haploids from flint genotypes are related to dominant genes existing in maize, *C1-I*, *C2-Idf* and *In1-D*, which inhibit anthocyanin synthesis of the marker for the selection of haploids (Coe 1994). When there is at least one of the genes present in a maternal genotype, the isolation of haploids is very difficult due to a lack of pigmentation of endosperm and embryo. Genes, that inhibit anthocyanin synthesis, are rather rare in dent maize. Therefore, anthocyanin marker genes in dent maize mostly are well expressed (Röber 1999). Previously, we supposed that the inhibitor genes of anthocyanin production exist in flint maize at a much higher rate than in dent maize. Therefore it was assumed that there would be a problem to select haploids from flint maize. The same was expected for flint×dent hybrids. Haploids were obtained from all maternal genotypes included in the experiment. The expression of the *R1-nj* gene in embryos of flint maize was even better than in dent and flint×dent genotypes. This is very important, since flint lines have a significant value for maize breeding in Central Europe. For commercial purposes it is necessary to develop homozygous lines from both dent and flint maize.

To a certain extent seeds produced by pollination with pollen of a haploid-inducing line did not produce any embryos. We suppose that embryo-less seeds are the result of a failure in egg cell development without fertilization. Furthermore, embryo-less seeds can be the result of the existence of lethal mutant genes in the maternal genotype, from which haploids were obtained. In this case, embryo-less seeds can reflect the frequency of lethal mutant genes existing in the maternal genotype.

After producing haploids, the next step is chromosome doubling in order to produce new homozygous lines. For this purpose two methods were tested. Both methods were effective and allowed us to obtain new doubled-haploid lines. Nevertheless, the method developed by Deimling et al (1997) was more convenient for use on a large scale due to lower labor requirements. Moreover, using this technique, very encouraging results were produced even under the unfavorable climatic conditions of the year 2000 in Freising and therefore it can be expected to gain even better results when weather conditions are optimal.

Haploid plants are a perfect tool for the rapid production of homozygous lines, and at the same time they can be used for an efficient improvement of quantitative traits important for breeding. Our tests showed that the selection of haploid plants, obtained from two synthetic populations, SP and SA, caused an increase of the grain yield and influenced some other traits of diploid plants. We suppose that this is the result of the following two factors. Firstly, the selection of large ears in haploid plants possibly increased the frequency of genes that have a significant contribution to plant vigor and plant viability. Secondly, haploid plants that have unfavorable harmful genes, either die or are weak and sterile and do not form seeds. Thus, the frequency of harmful genes is reduced by means of natural selection. Haploid plants can therefore be considered as a natural filter that discards harmful genes from a population, or any other breeding material, from which haploids were obtained. We suppose that clearing our synthetic populations from harmful genes leads to a positive effect on some plant and ear traits. Even some traits were improved which were not selected during HSRS.

Thus, we conclude that all the elements of the in vivo haploid inducing technology tested were effective. The utilization of maternal haploid plants has great potential for maize breeding and maize genetics.

References

Bylich VG, Chalyk CT (1996) Existence of pollen grains with a pair of morphologically different sperm nuclei in the ZMS line. Maize Genet Coop Newslett 70:33

Chalyk ST (1999) Creating new haploid-inducing lines of maize. Maize Genet Coop Newslett 73:53–54

Chalyk ST (Cealic ST), Rotarenco VA (1999) Using maternal haploid plants in recurrent selection in maize. Maize Genet Coop Newslett 73:56–57

Chase SS (1969) Monoploids and monoploid derivatives of maize (*Zea mays* L.). Bot Rev 135:117–167

Coe EH (1959) A line of maize with high haploid frequency. Am Nat 93:381–382

Coe EH (1994) Anthocyanin genetics. In: Freeling M, Walbot V (eds) The maize handbook. Springer-Verlag, New York, pp 279–281

Deimling S, Röber F, Geiger HH (1997) Methodik und Genetik der in-vivo-Haploideninduktion bei Mais. Vortr Pflanzenzüchtung 38:203–204

Enaleeva NKh, Tyrnov VS, Selivanova LP, Zavalishina AN (1996) Single fertilization and the problem of haploidy induction in maize. Doklady Biol Sci 353:225–226

Forughi-Wehr B, Wenzel G (1993) Andro- and partheno-genesis. In: Hayward MD, Bosemark NO, Romagosa I (eds) Plant breeding: principles and prospects. Published by Chapman and Hall, London, pp 261–277

Hallauer AR, Miranda JB (1988) Quantitative genetics in maize breeding, 2nd edn. Iowa State University Press, Ames, Iowa

Mahendru A, Sarkar KR (2000) Cytological analysis of the pollen of haploidy inducer lines in maize. Indian J Genet Plant Breed 60:37–43

Nanda DK, Chase SS (1966) An embryo marker for detecting monoploids of maize (*Zea mays* L.). Crop Sci 6:213–215

Röber F (1999) Fortpflanzungsbiologische und genetische Untersuchungen mit RFLP-Markern zur in-vivo-Haploideninduktion bei Mais. Phd Thesis, Universität Hohenheim

Tyrnov VS, Zavalishina AN (1984) Inducing high frequency of matroclinal haploids in maize (in Russian). Dokl Akad Nauk SSSR 276:735–738

Zabirova ER, Chumak MV, Shatskaia OA, Scherbak VS (1996) Technology of the mass accelerated production of homozygous lines (in Russian). Kukuruza i sorgo N 4:17–19