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Inheritance of Glutenin Subunits in F1 Seeds of Reciprocal Crosses Between European Hexaploid Wheat Cultivars

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Summary. Ten pairs of reciprocal crosses have been made between wheat cultivars which show differences in their glutenin subunit compositions. The F1 seed glutenin subunit composition was studied by means of polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS). The results indicate that all the high molecular weight (HMW) and medium molecular weight (MMW) subunits (from 133,000 to 65,000 daltons) are transmitted to the F1 seed generation from the parental cultivars. In accordance with the triploid nature of the heterozygous endosperm (3n) and with the maternal and paternal gene dosage ratio (2 : 1) in the endosperm itself, a significant effect of maternal parent is registered when comparing pairs of reciprocal seeds. Genes coding for the glutenin subunits are expressed whatever their doses are (one, two, or three) in the hybrid endosperm; thus the glutenin subunits inheritance is consistent with the co-dominant type.

For one pair of the reciprocal crosses, two MMW parental bands (MW: 71,000 and 66,000) seemed absent in the F1 seed patterns while a new band with an intermediate, apparent MW (68,000) appears. This phenomenon was observed when the glutenins analyzed by electrophoresis were previously separated from other endosperm proteins, and not when they were directly extracted from the ground seed. We assume that the extraction can cause interactions between moieties attached to the subunits and lead to the formation of a complex having an intermediate electrophoretic mobility.

Key words: Glutenin – Wheat – Co-dominance – Bread-making

Introduction

Cereal endosperm proteins which are isolated from varieties within different species evidence electrophoretic heterogeneity and variability.

This has been extensively investigated for prolamins, the 70% ethanol soluble protein fraction, in oats (Kim and Mossé 1979), corn (Gentinetta et al. 1975), and barley (Scriban and Strobbel 1978; Shewry et al. 1978a, 1978c; Doll and Brown 1979; Shewry et al. 1979) as well as in wheat varieties (Autran 1973; Autran and Bourdet 1975; Wrigley and Shepherd 1974; Damidaux et al. 1978; Zillman and Bushuk 1979; Tkachuk and Metlish 1980). Research on glutenin, more precisely defined as the protein fraction not soluble in 70% ethanol and excluded during gel filtration on Sephadex G-100 in the presence of acidic ionic detergents (Kaczkowski and Tkachuk 1980), have been performed essentially for determining their variability in hexaploid wheat varieties (Orth and Bushuk 1973; Bietz et al. 1975; Payne et al. 1979; Bietz and Huebner 1980; Burnouf and Bouriquet 1980; Lawrence and Shepherd 1980). Glutenins from a wide range of genotypes differ, often greatly, in number and molecular weight of subunits.

Genes controlling wheat glutenin subunit synthesis have been located (Orth and Bushuk 1974; Bietz et al. 1975; Payne et al. 1980b; Brown and Flavell 1981), and the existence of allelic subunits has been demonstrated (Payne et al. 1980a, 1980b, 1981b; Lawrence and Shepherd 1981). However, to our knowledge, no studies have examined glutenin subunits inheritance in progeny of reciprocal crosses. Since glutenin is polymorphic, electrophoresis may reveal significant features of the transmission and expression of glutenin-coding genes from parental varieties to progeny. This knowledge is important in early selection of bread-making varieties since glutenin is considered to be the prime factor governing the dough strength and elasticity (Wall 1979), and since relationships between electrophoretically resolved glutenin subunits and the bread-making quality, though not always obvious (Orth and Bushuk 1973; Bietz et al. 1975; Lawrence and Shepherd 1980) have been found in European varieties (Burnouf and Bouriquet 1980) and proved to exist in progeny of crosses (Payne et al. 1979, 1980a, 1981a).

For these reasons, we made reciprocal crosses between hexaploid wheat cultivars, chosen in accordance with our previous results, having different bread-making quality and diverse glutenin subunit composition. We here report on the inheritance of glutenin subunits in the F1 seed generation.

Materials and Methods

Crosses

Reciprocal crosses have been performed between (1) wheat cultivars showing very good French bread-making quality

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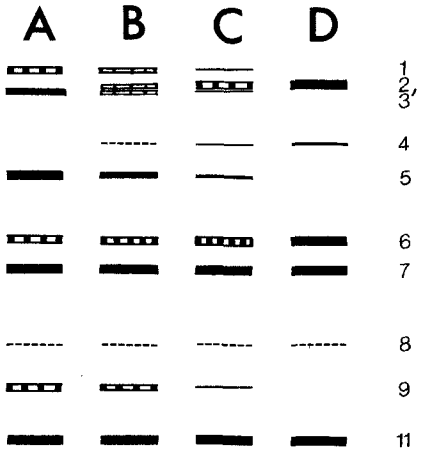

Fig. 1A–D. Diagram of HMW and MMW glutenin subunits from parental cultivars **A** 'Gamin'; **D** 'Maris-Huntsman'; and from reciprocal F1 seeds **B** 'Gamin'×'Maris-Huntsman'; **C** 'Maris-Huntsman'×'Gamin'

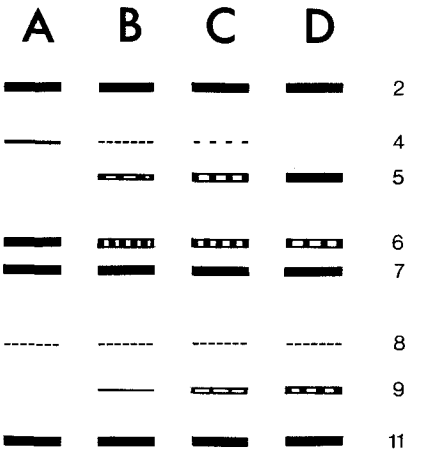

Fig. 3A–D. Diagram of HMW and MMW glutenin subunits from parental cultivars **A** 'Clement', 'Maris-Huntsman'; **D** 'Capitole', 'Rudi'; and from reciprocal F1 seeds **B** 'Clement'× 'Capitole', 'Maris-Huntsman'×'Capitole', 'Clement'×'Rudi'; **C** 'Capitole'×'Clement', 'Capitole'×'Maris-Huntsman', 'Rudi' ×'Clement'

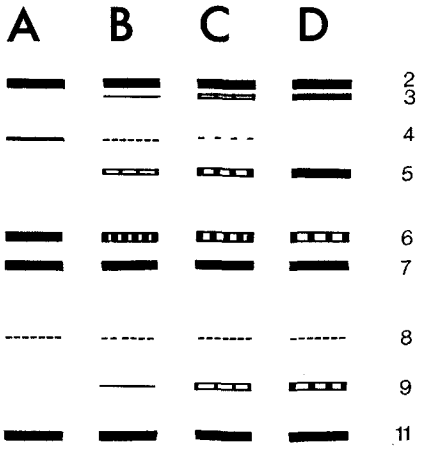

Fig. 2A–D. Diagram of HMW and MMW glutenin subunits from parental cultivars **A** 'Clement', 'Maris-Huntsman'; **D** 'Hardi'; and from reciprocal F1 seeds **B** 'Clement'×'Hardi', 'Maris-Huntsman'×'Hardi'; **C** 'Hardi'×'Clement', 'Hardi'× 'Maris-Huntsman'

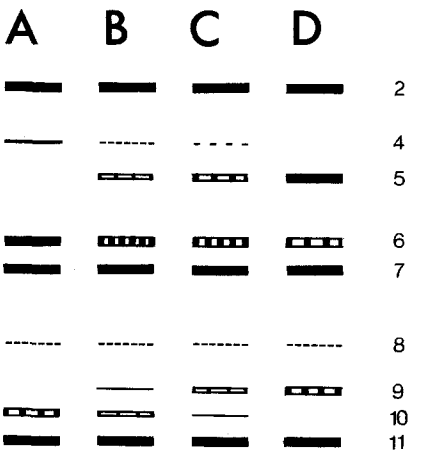

Fig. 4A–D. Diagram of HMW and MMW glutenin subunits from parental cultivars **A** 'Corin', 'Joss'; **D** 'Capitole', 'Rudi'; and from reciprocal F1 seeds **B** 'Corin'×'Capitole', 'Joss'× 'Capitole', 'Joss'×'Rudi'; **C** 'Capitole'×'Corin', 'Capitole'× 'Joss', 'Rudi'×'Joss'

('Hardi', 'Capitole' and 'Rudi') or medium French bread-making quality ('Gamin') and (2) cultivars of mediocre French bread-making quality ('Joss' and 'Corin') or cultivars considered unsuitable for French bread-making ('Maris-Huntsman' and 'Clement'). Crosses were made using standard conditions of emasculations and hand-pollinations. Ten pairs of reciprocal crosses were studied: 'Hardi'-'Clement'; 'Hardi'-'Corin'; 'Hardi'-'Maris-Huntsman'; 'Capitole'-'Clement'; 'Capitole'-'Corin'; 'Capitole'-'Joss'; 'Capitole'-'Maris-Huntsman'; 'Rudi'-'Clement'; 'Rudi'-'Joss'; 'Gamin'-'Maris-Huntsman'.

Preparation of Glutenin and SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

For each of the crosses, 5 to 12 half F1 seeds from two different plants were analyzed separately. Glutenin was prepared after sequential extractions of albumin, globulin and gliadin from 15 to 20 mg of undefatted ground seed (Bietz et al. 1975). In addition, the same quantity of flour was directly suspended in electrophoretic sample buffer and glutenin subunits were analyzed (Payne et al. 1979). The electrophoretic method was used before (Burnouf and Bouriquet 1980), except that we used 7% acrylamide to improve band sharpness. Gels were stained with Coomassie Brilliant Blue R 250 and scanned at 588 nm (orange filter N° 16) using a PHI Vernon integrator densitometer.

Results

Differences in the glutenin subunit composition from the parental cultivars were essentially found among

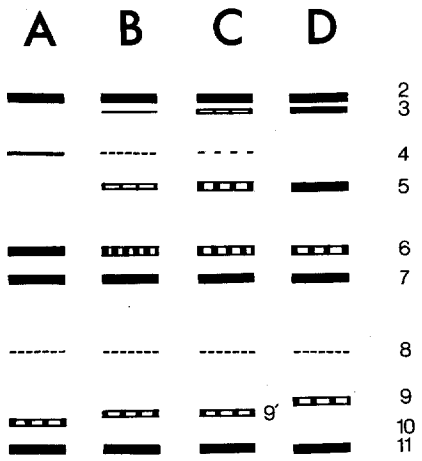

Fig. 5A–D. Diagram of HMW and MMW glutenin subunits from parental cultivars **A** 'Corin'; **D** 'Hardi'; and from reciprocal F1 seeds **B** 'Corin'×'Hardi'; **C** 'Hardi'×'Corin'. Analysis performed after sequential extraction of glutenins

polypeptides having MWs more than 62,000 daltons (bands 1, 2, 3, 3′, 4, 5, 9, and 10) as previously shown by SDS-PAGE (Burnouf and Bouriquet 1980).

The results that we have obtained in studying the reciprocal crosses (except the pair of crosses between 'Hardi' and 'Corin') are presented in Figs. 1–4. The composition in glutenin subunits of the parental cultivars is shown in the patterns A and D, those of the reciprocal F1 hybrids in the patterns B (cross A×D) and C (cross D×A). The analyses indicate that the reciprocal F1 seeds qualitatively present the same glutenin subunit composition. Indeed, all the HMW and MMW parental glutenin subunits were transmitted to the F1 generation. The components which showed the same mobility and which were synthesized at identical concentrations in the parental patterns (e.g. bands 2 and 7, Figs. 2 and 3, respectively) were present in the F1 hybrid patterns as a similar concentration. The bands in the patterns that were unique to one or the other parent of the reciprocal crosses were also detected in the F1 seed patterns; however, their syntheses were influenced by the direction of the cross (e.g. band 1, Fig. 1; band 3, Fig. 2; bands 4 and 5, Fig. 3; bands 9 and 10, Fig. 4). Indeed, densitometry indicated that the staining of such bands was reduced to about 2/3 in the F1 hybrid seed patterns when these bands were only present in the female parent and about 1/3 when detected only in the male. The amount of a protein component synthesized in the endosperm appeared to be proportional to the number of copies of the structural gene coding for that component; these results are in accordance with the gene dosage levels reflecting the maternal : paternal cultivars genome ratio of 2 : 1 in the triploid hybrid endosperm tissue. So, the subunit composition of glutenin from pairs of reciprocal F1 seeds is quantitatively influenced to a greater extent by that of the female.

Electrophoretic analyses of the protein fraction prepared by differential solvent extraction from the seeds of reciprocal hybrids between 'Hardi' and 'Corin' (Fig. 5) showed the presence of a band (called 9′) not detected in the parents. This band had an apparent MW (68,000) between that of band 9 (in 'Hardi'; MW 71,000) and band 10 (in 'Corin'; MW 66,000) which were not detected in the hybrid patterns. Its intensity appeared to be similar in the reciprocal seeds and unrelated to the direction of the cross. In contrast, when the analyses were made on the ground seed directly suspended in the sample buffer, the electrophoretic patterns indicated that bands 9 and 10 were inherited in the hybrids.

Discussion

Our observations lead us to conclude that the inheritance of the glutenin subunits studied here is consistent with the co-dominant type. Indeed, the transmission of glutenin subunits to F1 progeny from the parental generation is of the same type as for prolamin components. Studies on gliadins (Mecham et al. 1978; Autran 1979; Sozinov and Poperelya 1980), hordeins (Shewry et al. 1978 b) and avenins (Kim et al. 1979), for example, show that the synthesis of these prolamins in the F1 hybrid reflects a gene dosage effect consistent with the existence of double fertilization in angiosperms leading to the development of the large triploid endosperm tissue in cereals. So, the patterns of F1 seeds show all protein components in the parental electrophoregrams, but the staining intensity of a protein in the pattern that is unique to one parent is related to the direction of the cross. Furthermore, we observed that the genes coding for HMW and MMW glutenin subunits are expressed in the hybrid endosperm regardless of their dosage. This was also noted for prolamins by the above cited authors.

The reason for presence of the band 9′ in the subunit pattern of glutenin extracted from the reciprocal F1 hybrids between 'Hardi' and 'Corin' remains to be determined. Regarding our experimental conditions, we should stress the following point: after differential solvent extraction of glutenin prior to the electrophoretic analysis, the staining of band 9′ was apparently similar whatever was the direction of the cross, and consequently did not evidence a gene dosage effect. This favors an electrophoretic co-migration of two (or more) different polypeptides coded by both parental genomes and not by only one. Taking this point into account and assuming that contaminating proteins did not mask the detection of the electro-

phoretic migrations of bands 9 and 10, we are of the opinion that the observed phenomenon is inadequately explained by: (1) possible interactions between the gene of 'Hardi' coding for the band 9 and that of 'Corin' coding for the band 10; (2) a process of an 'in vivo' cutting (Burr et al. 1978) of band 9 by an enzyme synthesized by 'Corin' that modifies its MW and, consequently, its electrophoretic mobility. On the other hand, the presence of moieties attached to the glutenin subunits has already been proposed (Lawrence and Shepherd 1980); we suggest that some interactions between a moiety attached to subunit 9 and another one attached to subunit 10 can occur during the extraction process and lead to the formation of a complex having an intermediate mobility. This was not observed while studying F1 hybrids from crosses between 'Capitole' and 'Corin' although 'Capitole' possesses band 9. We assume, therefore, that the band 9 in 'Capitole' and in 'Hardi' are of different nature. However, we must point out that the explanation we propose remains speculative until further studies prove its reality. Furthermore, the glutenin nature of one or more of these bands remains to be confirmed since the presence of albumins, globulins, and gliadins in that range of MW is possible (Payne and Corfield 1979; Brown and Flavell 1981), especially when the electrophoresis is performed directly on the total protein extracts of the ground seed.

Glutenin and gliadin composition of F1 seeds is influenced by a maternal gene dosage effect. This feature may be of importance for wheat breeding to improve bread-making quality, as previously noted by Bernacka et al. (1977) and Filutowicz et al. (1978) who studied the inheritance of the fractional composition of seed proteins in the F1 generation. Since the reciprocal F1 embryos have the same set of the parental chromosomes, further investigations should be carried out to confirm this maternal influence in subsequent generations. Studies of the subunit composition of glutenin from reciprocal F2 seeds should better reveal the genetics of glutenin subunit transmission.

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Literature

Autran, J.C. (1973): L'identification des variétés de blé. Bull. Anc. Elèves Ecole Francaise de Meunerie **256,** 163–169

Autran, J.C. (1979): Déterminisme génétique de certains composants des gliadines. Journée sur la 'biochimie-génétique' du blé. Paris, 24 avril

Autran, J.C.; Bourdet, A. (1975): L'identification des variétés de blé: établissement d'un tableau général de détermination fondé sur le diagramme électrophorétique des gliadines du grain. Ann. Amélior. Plant. **25,** 277–301

Bernacka, T.; Kaczkowski, J.; Liss, W.; Serwatowska, J. (1977): The fractional composition of wheat proteins and the possibility of its hereditability in F1 generation. Acta Agrobot. **30,** 5–11

Bietz, J.A.; Huebner, F.R. (1980): Structure of glutenin: achievements at the Northern Regional Research Center. Ann. Tech. Agric., Workshop on the physico-chemical properties of wheat gluten proteins. Nantes (France) **29,** 249–277

Bietz, J.A.; Shepherd, K.W.; Wall, J.S. (1975): Single-kernel analysis of glutenin: use in wheat genetics and breeding. Cereal Chem. **52,** 513–532

Brown, J.W.S.; Flavell, R.B. (1981): Fractionation of wheat gliadin and glutenin subunits by two-dimensional electrophoresis and the role of group 6 and group 2 chromosomes in gliadin synthesis. Theor. Appl. Genet. **59,** 349–359

Burnouf, T.; Bouriquet, R. (1980): Glutenin subunits of genetically related European hexaploid wheat cultivars: their relation to bread-making quality. Theor. Appl. Genet. **58,** 107–111

Burr, B.; Burr, F.A.; Rubenstein, I.; Simon, M.N. (1978): Purification and translation of zein messenger RNA from maize endosperm protein bodies. Proc. Natl. Acad. Sci. USA **75,** 696–700

Damidaux, R.; Autran, J.C.; Grignac, P.; Feillet, P. (1978): Relation applicable en sélection entre l'électrophorégramme des gliadines et les propriétés visco-élastiques du gluten de *Triticum durum* Desf. C.R. Acad. Sci. D **287,** 701–704

Doll, H.; Brown, A.H.D. (1979): Hordein variation in wild (*Hordeum spontaneum*) and cultivated (*Hordeum vulgare*) barley. Can. J. Genet. Cytol. **21,** 391–404

Filutowicz, H.; Serwatowska, J.; Kaczkowski, J. (1978): Heredity of the fractional composition of wheat grain proteins in the F1 generation. Acta Soc. Bot. Polon. **47,** 269–278

Gentinetta, E.; Maggiore, T.; Salamini, F.; Lorenzoni, C.; Pioli, F.; Soave, C. (1975): Protein studies in 46 "opaque 2" strains with modified endosperm texture. Maydica **20,** 145–164

Kaczkowski, J.; Tkachuk, R. (1980): Résumé des sessions. Session IV. Ann. Techn. Agric., Workshop on the physico-chemical properties of wheat gluten proteins. Nantes (France) **29,** 107–108

Kim, S.I.; Mossé, J. (1979): Electrophoretic patterns of oat prolamins and species relationships in *Avena.* Can. J. Genet. Cytol. **21,** 309–318

Kim, S.I.; Saur, L.; Mossé, J. (1979): Some features of the inheritance of avenins, the alcohol soluble proteins of oat. Theor. Appl. Genet. **54,** 49–54

Lawrence, G.J.; Shepherd, K.W. (1980): Variation in glutenin protein subunits of wheat. Aust. J. Biol. Sci. **33,** 221–233

Lawrence, G.J.; Shepherd, K.W. (1981): Inheritance of glutenin protein subunits of wheat. Theor. Appl. Genet. **60,** 333–337

Mecham, D.K.; Kasarda, D.D.; Qualset, C.O. (1978): Genetic aspects of wheat gliadin proteins. Biochem. Genet. **16,** 831–853

Orth, R.A.; Bushuk, W. (1973): Studies of glutenin. II. Relation of variety, location of growth, and baking quality to molecular weight distribution of subunits. Cereal Chem. **50,** 191–197

Orth, R.A.; Bushuk, W. (1974): Studies of glutenin. VI. Chromosomal location of genes coding for subunits of glutenin of common wheat. Cereal Chem. **51,** 118–126

Payne, P.I.; Corfield, K.G. (1979): Subunit composition of wheat glutenin proteins, isolated by gel filtration in a dissociating medium. Planta **145,** 83–88

Payne, P.I.; Corfield, K.G.; Blackman, J.A. (1979): Identification of a high-molecular-weight subunit of glutenin whose presence correlates with bread-making quality in wheats of related pedigree. Theor. Appl. Genet. **55,** 153–159

Payne, P.I.; Corfield, K.G.; Holt, L.M.; Blackman, J.A. (1981a): Correlations between the inheritance of certain high molecular weight subunits of glutenin and bread-making quality in progenies of six crosses of bread wheat. J. Sci. Food Agric. **32,** 51–60

Payne, P.I.; Harris, P.A.; Law, C.N.; Holt, L.M.; Blackman, J.A. (1980a): The high-molecular-weight subunits of glutenin: structure, genetics, and relationship to bread-making quality. Ann. Techn. Agric., Workshop on the physicochemical properties of wheat gluten proteins. Nantes (France) **29,** 309–320

Payne, P.I.; Holt, L.M.; Law, C.N. (1981b): Structural and genetical studies on the high molecular weight subunits of wheat glutenin. Part I: Allelic variation in subunits amongst varieties of wheat (*Triticum aestivum*). Theor. Appl. Genet. **60,** 229–236

Payne, P.I.; Law, C.N., Mudd, E.E. (1980b): Control by homoeologous group 1 chromosomes of the high-molecular-weight subunits of glutenin, a major protein of wheat endosperm. Theor. Appl. Genet. **58,** 113–120

Scriban, R.; Strobbel, B. (1978): Etude chimiotaxonomique de l'orge et du malt par électrofocalisation sur gel. C.R. Acad. Sci. D **287,** 841–843

Shewry, P.R.; Ellis, J.R.S., Pratt, H.M.; Miflin, B.J. (1978a): A comparison of methods for the extraction and separation of hordein fractions from 29 barley varieties. J. Sci. Food Agric. **29,** 433–441

Shewry, P.R.; Pratt, H.M.; Faulks, A.J.; Parmar, S.; Miflin, B.J. (1979): The storage protein (hordein) polypeptide pattern of barley (*Hordeum vulgare* L.) in relation to varietal identification and disease resistance. J. Natl. Inst. Agric. Bot. **15,** 34–50

Shewry, P.R.; Pratt, H.M.; Finch, R.A.; Miflin, B.J. (1978b): Genetic analysis of hordein polypeptides from single seeds of barley. Heredity **40,** 463–466

Shewry, P.R.; Pratt, H.M.; Miflin, B.J. (1978c): Varietal identification of single seeds of barley by analysis of hordein polypeptides. J. Sci. Food Agric. **29,** 587–596

Sozinov, A.A.; Poperelya, F.A. (1980): Genetic classification of prolamines and its use for plant breeding. Ann. Techn. Agric., Workshop on the physicochemical properties of wheat gluten proteins. Nantes (France) **29,** 229–245

Tkachuk, R.; Metlish, V.J. (1980): Wheat Cultivar identification by high voltage gel electrophoresis. Ann. Techn. Agric., Workshop on the physico-chemical properties of wheat gluten proteins. Nantes (France) **29,** 207–212

Wall, J.S. (1979): The role of wheat proteins in determining backing quality. In: Recent advances in the biochemistry of cereal (eds. Laidman, D.L.; Wyn Jones, R.G.), pp. 275–311. London, New York, San Francisco: Academic Press

Wrigley, C.W.; Shepherd, K.W. (1974): Identification of Australian wheat cultivars by laboratory procedures. Examination of pure samples of grain. Aust. J. Exp. Agric. Anim. Husb. **14,** 796–804

Zillman, R.R., Bushuk, W. (1979): Wheat cultivar identification by gliadin electrophoregrams. III. Catalogue of electrophoregram formulas of Canadian wheat cultivars. Can. J. Plant Sci. **59,** 287–298

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