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[54] **BUFFALOGRASS PLANT NAMED 'NE86-61'**

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[52] **U.S. Cl.** **Plt./391**

[58] **Field of Search** **Plt./90, 391**

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[57] **ABSTRACT**

A vegetatively reproduced buffalograss cultivar named NE86-61 is distinguished from other commercially produced buffalograss varieties by its excellent turfgrass color, cold tolerance, high density, dark green color, low growth habit, and rate of establishment. NE86-61 is also distinguished from other varieties by molecular markers and nuclear DNA content. NE86-61 is suitable for use in low to medium maintenance situations in arid and semi-arid climates of United States and Canada.

3 Drawing Sheets

1

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e) to U.S. provisional application Ser. No. 60/028,749, filed Oct. 23, 1996. This application is also related to U.S. provisional application Ser. No. 60/028,987, filed Oct. 22, 1996, and U.S. provisional application Ser. No. 60/028,988, filed Oct. 22, 1996, both hereby incorporated by reference in their entireties. This application is also related to U.S. plant

2

application Serial No. 08/955,786 and U.S. plant application Ser. No. 08/956,070, both filed on even date herewith, both hereby incorporated by reference in their entireties.

BACKGROUND OF THE INVENTION

Buffalograss [*Buchloë dactyloides* (Nutt) Engelm.] is a perennial, low growing, dioecious, warm-season grass species native to the Great Plains of North America. It thrives in semi-arid conditions, even under heavy grazing pressure,

5

and spreads by branching stolons, creating a dense sod (Wenger, 1943; Beetle, 1950; Huff & Wu, 1987). Because of this adaptation, buffalograss can withstand combinations of cold, heat, and drought stress, yet still maintain an attractive turf (Wenger, 1943). The center of origin for buffalograss is most likely central Mexico (Quinn & Engel, 1986; Shaw et al., 1987). It is native to an area from central Mexico to southern Canada (Wenger, 1943; Beetle, 1950) and altitudes below 2000 meters (Beetle, 1950). The range of adaptation is relatively stable, however seasonal precipitation variation may alter the eastern boundary (Wenger, 1941; 1943; Beetle, 1950). Buffalograss is the dominant species in the short grass prairie and cannot compete with the taller grasses in prairie communities in higher rainfall areas (Poransky, 1983). Buffalograss was threatened with extinction due to heavy grazing and agricultural production (Beetle, 1950), but during the 1930's, it was recognized for its usefulness in restoring plant cover in the Great Plains after extreme drought, to prevent wind and water erosion of the soil. The drought resistance of buffalograss has been shown, in part due to its low evapotranspiration rate of six mm per day under optimum growth conditions. This is less than any other commonly used warm and cool-season turfgrass. Characteristics responsible for the drought resistance include a finley branched root system, low growth habit, and the ability of leaf blades to limit transpiration by rolling longitudinally during drought stress (Savage & Jacobsen, 1935; Engleke & Hickey, 1983). Buffalograss will go dormant sooner than other grasses will resume growth quickly once favorable moisture returns (Savage & Jacobsen, 1935; Beetle, 1950). Buffalograss survived mowing heights under 2.5 inches better than other native species with a noticeable increase in horizontal spreading and improved weed competition. These drought resistant and mowing tolerant characteristics make buffalograss a useful turfgrass in semi-arid portions of North America. Buffalograss can be established by two methods: vegetative propagation or seed (Wenger, 1943; Poransky, 1983). Buffalograss establishment has typically been expensive. Vegetative propagation of buffalograss plugs or sod pieces has traditionally been done because low seed production of native stands and poor seedling establishment. Developments of automated pluggers and "big roll" sod handlers (Riordan et al., 1993) have made vegetative propagation more economical. Improvements have also been made in seed production and seed treatments (Klingenberg, 1993). Buffalograss has been used for many years on highway shoulders or right-of-ways, airfield runways, cemeteries, parks, golf courses, and other athletic fields (Wenger, 1943; Beetle, 1950; Poransky, 1983). Because of environmental concerns and changes in landscape priorities, buffalograss has tremendous potential as turfgrass. Breeding and development efforts are relatively new with emphasis on developing turf-type buffalograss cultivars which have low growth habit, improved color, faster establishment, improved density, extended growth season, and recuperative potential (Riordan et al., 1993; Engleke & Lehman, 1990; Wu & Harivandi, 1991).

SUMMARY OF THE INVENTION

'NE86-61' is distinguished from other commercially available buffalograss cultivars. It is a vegetatively propagated female clone with darker green (nearly blue-gray-green) foliage and lower growth habit than 'Prairie,' '609,' '315,' and newly introduced 'NE86-120' and 'NE91-118' buffalograsses. 'NE86-61' also exhibits better overall turfgrass quality, density, and uniformity than seeded buffa-

lograss varieties. It is best adapted to central and northern parts of the Great Plains. 'NE86-61' provides an attractive, wear tolerant turf which requires less water, fertilizer, and mowing than other turfgrass species. 'NE86-61' is also tolerant to relatively low mowing heights because of its low growth habit. These characteristics, along with field and greenhouse evaluations have shown 'NE86-61' is well adapted to golf course fairways and roughs, home lawns, and general use areas requiring reduced management inputs.

BRIEF DESCRIPTION OF THE ILLUSTRATIONS

FIG. 1 is a photograph of a typical stolon of 'NE86-61'.

FIG. 2 is a photograph of turf produced by 'NE86-61', taken at Mead, Nebr., showing darker green color, uniformity, and female flowering.

FIG. 3 is a photograph of 'NE86-61', at Mead, Nebr., exhibiting high turfgrass quality when mowed at $\frac{5}{8}$ ".

FIG. 4 depicts DNA fingerprints of selected buffalograss varieties.

DETAILED DESCRIPTION OF THE PLANT

Buffalograss NE86-61 was found in a mowed and maintained (cultivated) rest area, east of Kensington, Smith County, Kans. After being collected, it was vegetatively (asexually) propagated. This is the way all buffalograsses are handled if they are being clonally propagated. In particular, buffalograss 'NE86-61' was propagated vegetatively (asexually) at the John Seaton Anderson Turfgrass Research Facility near Mead, Nebr. Plugs and stolons were used to propagate it. The cultivar is completely stably reproduced by the aforementioned means. This selection, along with several thousand other selections, was evaluated at the John Seaton Anderson Turfgrass Research Facility near Mead, Nebr. The growth characteristics, turfgrass evaluation ratings, and molecular markers can be used to distinguish NE-86-61 from other commercially produced buffalograss varieties.

Morphological Characteristics

'NE-8661' is noteworthy because of its low growing height and color. Although canopy height is lower than most other buffalograsses, it is not dwarf in other respects. Buffalograss 'NE86-61' internodes are slightly larger than '315' and 'NE91-118', and shorter than '609,' 'Texoka,' and 'NE86-120' (Table 1). Stolon node width of 'NE86-61' is smaller than the other selections, with the exception of, perhaps, 'NE91-118'. Like other turf varieties '315,' '609,' 'NE86-120', and 'NE91-118', leaf measurements are smaller than 'Texoka' (Table 2), which result in a turf of finer texture (FIG. 1). Pubescent leaves (Table 4) are a distinguishing characteristic of 'NE86-61'. The purpose or adaptation of pubescence on buffalograss is not known, but may decrease evaporation of water from leaf surfaces (Kramer, 1983). The size of the female flowers (burs) is similar to other varieties (Table 3).

Turfgrass Characteristics

A quality turfgrass stand must have good, pleasing color. 'NE86-61' has consistently produced darker green turf which may best be described as a gray-blue-green color (FIG. 2). Darker turf color is usually favored by most consumers. Turfgrass color ratings of 'NE86-61' are higher in greenhouse evaluations, compared to '315' U.S. Plant Pat. No. 9,847 '609' U.S. Plant Pat No. 8,475 'Texoka,' 'NE86-

120', and 'NE91-118' (Table 5). 'NE86-61' also produces the best color ratings in field evaluations (Tables 6, 7, & 8). Turfgrass quality is a combination of several factors: density, texture, uniformity, and color (Turgeon, 1980). 'NE86-61' has much improved quality compared to older, forage type buffalograsses such as 'Texoka,' but also improved quality compared to turf varieties '315,' '378,' '609,' and 'Prairie' U.S. Plant Pat. No. 7,539 (Tables 6, 7, 8). 'NE86-61' also exhibited superior quality when mowed at heights down to $\frac{5}{8}$ ", similar to the height used on golf course fairways (Table 6; FIG. 3). Lower growth habit and denser canopy contribute to its tolerance to low mowing. 'NE86-61' has a moderate rate of establishment compared to other buffalograsses. It is similar to '315,' slower than 'Texoka,' but faster than '378' (Table 8). In this trial, vegetative types were slower to establish than seeded varieties, however, plug spacing at planting has a major effect on plot cover in the first year of any planting (Johnson et al., 1997).

Buffalograss 'NE86-61' cannot at this time be distinguished from other buffalograsses based on the characteristics of heat, drought, or salinity tolerance. Buffalograss has better heat and drought tolerance than other turfgrass species, but less salinity tolerance.

Buffalograss 'NE86-61' has canopy density and inflorescence characteristics that are not distinguishable from other buffalograsses, with the exception of, perhaps, 'Texoka.' Buffalograss canopy density and inflorescence characteristics would be different than for other species.

All northern adapted plants survive the winter cold period by going dormant early in fall and resuming growth in the spring (Riordan et al., 1993). 'NE86-61' is a typical northern-adapted cultivar in this respect. Southern adapted cultivars, such as '609' and 'Prairie,' remain actively growing late into the fall, resulting in higher quality ratings (Tables 6, 7, & 8) but are subject to winterkill during Nebraska winters.

Molecular Marker Analysis

DNA fingerprinting was conducted using polymerase chain reaction (PCR) (Welsh and McClelland, 1991; Williams et al., 1990). DNA profiles of 'NE86-61' were compared with '315,' '378,' NE-84-45-3, '609,' 'NE86-120-2', 'NE91-118', and bulked sample of 'Texoka' progeny. DNA was extracted from fresh leaf tissue using 'Easy DNA™ Kit' from Invitrogen (San Diego, Calif.). Fresh leaf tissue from each clone was ground with a mortar and pestle with liquid nitrogen. For 'Texoka,' leaf tissue was collected from three solid seeded pots and bulked to obtain a bulked DNA sample from this seeded cultivar. PCR was performed using decamer primers OPA-07 and OPA-16 from Operon Technologies (Alameda, Calif.). Samples were separated on a polyacrylamide gel and DNA fragments were visualized using ethidium bromide staining. DNA amplification was performed using an Idaho Technologies thermocycler (Idaho Falls, Id.) with two cycles of denaturing at 94° C. for one minute, annealing at 40° C. for seven seconds, and extension at 72° C. for 70 seconds, followed by 43 cycles of denaturing at 94° C. for three seconds, annealing at 40° C. for seven seconds, extension at 72° C. for 70 seconds. PCR reactions were performed in a volume of 25 μ l. The PCR reaction components were as follows:

Component	Volume	Concentration
Sterile H ₂ O	12.5 μ l	
10X buffer	2.5 μ l	
Tris		50 mM, pH 8.3
Bovine Serum Albumin		5 mg/ml
MgCl		25 mM
Sucrose		40% (w/v)
Cresol Red		1 mM
dNTPs	2.5 μ l	2 mM each dNTP
Taq DNA polymerase	2.5 μ l	0.4 U/ μ l
Primer	2.5 μ l	5 μ M
DNA	2.5 μ l	25 ng/ μ l

Primers used for DNA fingerprinting were OPA-07AND opa-16 from Operon Technologies. Samples were loaded in a 5% polyacrylamide gel and electrophoresed at 44 volts (24 milliamps) in a TBE-8 buffer for approximately 2 hours and 45 minutes. DNA was visualized in the gel using ethidium bromide staining. The DNA profiles produced are shown in FIG. 4. Using primer OPA-07, NE-8661 was the only genotype with a band > 2020 base-pairs (bp), but no band at 1150 bp. With primer OPA-16, NE-86-61 was the only genotype with bands at 390 bp, 710 bp, 1100 bp, but not at 560 bp.

Flow Cytometric Analysis

Flow cytometry was used to measure the nuclear DNA content within buffalograss cells. This method is a rapid way to differentiate between varieties having different numbers of chromosomes, and sometimes can differentiate among varieties having the same number of chromosomes. The detailed protocol for this method is listed below.

1. Collect young buffalograss leaves and keep moist at 4° C.
2. Place 25–30 mg of the buffalograss leaves and 25–30 mg of *Poa annua* (UM-184) leaves in a petri dish on ice. DNA content of UM-184 *Poa annua* was determined separately and repeatedly as 4.64 picograms of DNA/nucleus.
3. Add with 1.0 ml of ice-cold buffer-propidium iodide solution and chop leaves into thin strips (<0.5 mm) with a scalpel. 15 ml of buffer solution, enough for 12–13 samples contains 14.3 ml of MgSO₄ buffer solution (10mM MgSO₄·7H₂O, 50mM KCl, 5mM Hepes), 15 mg dithiothreitol, 300 μ l propidium iodide stock (5 mg/ml), 375 μ l Triton X-100 stock (10% w/v).
4. Filter the slurry through 30 μ m nylon mesh.
5. Centrifuge at 15,000 rpm for 15 seconds. Discard supernatant.
6. Resuspend in 200 μ l propidium iodide buffer solution + RNase. (3 ml of extraction solution and 7.5 μ l of DNAase free RNAase.).
7. Incubate sample for 15 min. at 37° C.
8. Analyze on a Becton-Dickson FACScan flow cytometer using a wavelength of 488 nm (FIG. 1).
9. Calculate DNA content of buffalograss sample.

$$= \frac{\text{mean of buffalograss peak}}{\text{mean of } Poa \text{ annua peak}} \times$$

DNA content of *Poa annua* (4.64 pg/nucleus)

Intact cell nuclei were isolated from young buffalograss leaves and passed through a flow cytometer to measure the amount of DNA in each nucleus. 'NE86-61' was determined to have 2.58 ± 0.02 picograms DNA per nucleus (Table 9). This places 'NE86-61' in the hexaploid group of buffalograsses together with '378' and 'NE86-120' having 60 chromosomes. 'Stampede' is a diploid having 20 chromosomes and 'Prairie,' '609,' and 'NE91-118' are tetraploid having 40 chromosomes. '315' is a pentaploid having 50 chromosomes.

Comparative Data

The following tables provide data comparison of 'NE86-61', 'NE86-120', 'NE91-118', '315,' '378,' '609,' 'Texoka,' and 'Prairie.'

TABLE 1

Internodal and nodal characteristics of buffalograsses (University of Nebraska Greenhouse, Winter, 1996-97).				
Selection	Internode Length ¹		Third Internode	Node
	Internode 2 (mm)	Internode 3 (mm)	Width ¹ (cm)	Pubescence ² (1-9)
'NE 86-61'	5.0 ± 1.5	4.7 ± 0.9	2.8 ± 0.4	6.2 ± 0.1
'NE 86-120'	5.3 ± 0.1	6.2 ± 0.4	3.9 ± 0.5	8.9 ± 0.1
'NE 91-118'	4.2 ± 0.3	4.4 ± 0.4	3.0 ± 0.4	1.2 ± 0.1
'315'	3.9 ± 0.6	3.7 ± 0.2	3.7 ± 0.4	5.7 ± 0.6
'609'	5.6 ± 0.1	6.0 ± 0.2	3.6 ± 0.4	1.0 ± 0.0
'Texoka'	6.1 ± 1.3	6.2 ± 2.0	4.9 ± 0.2	4.2 ± 0.8

¹Average of five measurements on each of three replications.

²Qualitative scale for trichome density, 1 = none, 5 = moderate, 9 = heavy or fuzzy

TABLE 2

Leaf length and width measurements of buffalograsses (University of Nebraska Greenhouse, Winter, 1996-97).		
Selection	Leaf blade Length ¹ (mm)	Leaf blade Width ¹ (mm)
'NE 86-61'	120.7 ± 1.8	1.6 ± 0.1
'NE 86-120'	165.3 ± 25.2	1.5 ± 0.1
'NE 91-118'	111.7 ± 6.4	1.4 ± 0.2
'315'	120.2 ± 9.2	1.6 ± 0.1
'609'	123.5 ± 5.1	1.4 ± 0.1
'Texoka'	198.9 ± 10.5	2.0 ± 0.1

¹Average of five measurements on each of three replications.

TABLE 3

Bur length and width measurements of buffalograsses (University of Nebraska Greenhouse, Winter, 1996-97).		
Selection	Bur Length ¹ (mm)	Bur Width ¹ (mm)
'NE 86-61'	7.0 ± 0.2	2.8 ± 0.7
'NE 86-120'	7.8 ± 0.6	2.8 ± 0.0
'315'	7.2 ± 0.2	2.5 ± 0.0
'609'	6.6 ± 0.2	3.0 ± 0.6
'Texoka'	7.3 ± 0.7	2.9 ± 0.4

¹Average of up to five measurements on each of three replications.

TABLE 4

Leaf pubescence ratings of buffalograsses (University of Nebraska Greenhouse, Winter, 1996-97).	
Selection	Leaf Pubescence ¹ (1-9)
'NE 86-61'	7.8 ± 0.1
'NE 86-120'	7.7 ± 0.2
'NE 91-118'	5.7 ± 0.4
'315'	5.5 ± 0.2
'609'	1.0 ± 0.0
'Texoka'	6.1 ± 0.4

¹Qualitative scale for trichome density, 1 = none, 5 = moderate, 9 = heavy or fuzzy.

TABLE 5

Genetic color ratings of buffalograsses (University of Nebraska Greenhouse, Winter, 1996-97).		
Selection	Genetic Color ¹ (1-9)	Leaf Color ²
'NE 86-61'	7.8 ± 0.2	137-A
'NE 86-120'	6.7 ± 0.9	137-A
'NE 91-118'	5.7 ± 0.4	137-C
'315'	6.2 ± 0.2	137-C
'609'	5.8 ± 0.2	137-C
'Texoka'	5.7 ± 0.7	137-C

¹Qualitative scale for plant color, 1 = yellow, 5 = medium green, 9 = dark green to bluegreen.

²According to the R.H.S. Colour Chart in association with the Flower Council of Holland; 137-A is darker than 137-C.

TABLE 6

Turfgrass quality, color, and fall dormancy ratings, 1992-96 ¹ (Area 16, University of Nebraska John Seaton Anderson Turfgrass Research Facility, Ithaca, NE).			
Selection	Turfgrass Quality ² (1-9)	Turfgrass Color ³ (1-9)	Fall Dormancy ⁴ (1-9)
'NE 86-61'	7.0	7.1	4.0
'NE 86-120'	6.7	6.3	3.9
'315'	6.4	5.8	3.9
'609'	4.5	5.7	6.7
'Prairie'	3.8	4.9	6.9
'Texoka'	4.6	5.4	4.9
LSD (.05)	0.7	0.7	1.4

¹Field plot was mowed at 2.5 inches during 1992 and 1993, at 5/8" during 1994-1996.

²Qualitative scale for overall quality, 1 = dead, 5 = acceptable quality, 9 = ideal turf quality.

³Qualitative scale for plant color, 1 = yellow, 5 = medium green, 9 = dark green to bluegreen.

⁴Qualitative rating of late fall growth, 1 = completely dormant, no green leaves visible, 5 = approx. 50% green leaves visible, 9 = no dormancy observed, all green leaves.

TABLE 7

Turfgrass quality, color, and fall dormancy ratings, 1994-96 (Area 17, University of Nebraska John Seaton Anderson Turfgrass Research Facility, Ithaca, NE).			
Selection	Turfgrass Quality ¹ (1-9)	Turfgrass Color ² (1-9)	Fall Dormancy ³ (1-9)
'NE 86-61'	6.6	6.2	5.6
'NE 86-120'	6.3	6.3	2.8
'NE 91-118'	5.8	5.3	5.3
'315'	6.1	5.8	4.9

TABLE 7-continued

Turfgrass quality, color, and fall dormancy ratings, 1994-96 (Area 17, University of Nebraska John Seaton Anderson Turfgrass Research Facility, Ithaca, NE).			
Selection	Turfgrass Quality ¹ (1-9)	Turfgrass Color ² (1-9)	Fall Dormancy ³ (1-9)
'378'	6.0	6.1	3.4
'Texoka'	4.9	4.8	4.2
LSD (.05)	1.0	1.1	2.4

¹Qualitative scale for overall quality, 1 = dead, 5 = acceptable quality, 9 = ideal turf quality.

²Qualitative scale for plant color, 1 = yellow, 5 = medium green, 9 = dark green to bluegreen.

³Qualitative rating of late fall growth, 1 = completely dormant, no green leaves visible, 5 = approx. 50% green leaves visible, 9 = no dormancy observed, all green leaves.

TABLE 8

Turfgrass quality, color, and fall dormancy ratings, 1995-96 (Area 25, University of Nebraska John Seaton Anderson Turfgrass Research Facility, Ithaca, NE).				
Selection	Turfgrass Quality ¹ (1-9)	Turfgrass Color ² (1-9)	Fall Dormancy ³ (1-9)	Establishment (8/20/95) (% cover)
'NE 86-61'	6.6	7.2	4.0	63.0
'315'	6.2	5.7	3.7	60.0
'378'	5.2	6.1	3.0	47.0
'Texoka'	4.9	4.4	5.3	70.0
LSD (.05)	1.0	1.0	1.4	13.0

¹Qualitative scale for overall quality, 1 = dead, 5 = acceptable quality, 9 = ideal turf quality.

²Qualitative scale for plant color, 1 = yellow, 5 = medium green, 9 = dark green to bluegreen.

³Qualitative rating of late fall growth, 1 = completely dormant, no green leaves visible, 5 = approx. 50% green leaves visible, 9 = no dormancy observed, all green leaves.

TABLE 9

DNA contents of buffalograss clones.			
Family name	n	DNA Content (pg/nucleus)	SE (pg/nucleus)
<u>Diploid¹</u>			
'Stampede'	7	0.93	0.01
<u>Tetraploid²</u>			
'Prairie'	2	1.80	0.00
'NE-91-118'	10	1.81	0.02
'609'	9	1.81	0.02
<u>Pentaploid³</u>			
'315'	11	2.29	0.02
<u>Hexaploid⁴</u>			
'NE 86-120'	24	2.59	0.04
'NE 86-61'	8	2.58	0.02
'378'	7	2.60	0.09

¹2n = 20

²2n = 40

³2n = 50

⁴2n = 60

The Variety

Origin: Plant selected from a mowed and maintained (cultivated) rest area, east of Kensington, Smith County, Kans.

Classification:

Botanic.—*Buchloë dactyloides* (Nutt.) Engelm.

Chromosome number: 2n=6x=60

Form: Monocot Gramineae.

Growth habit: A perennial plant, with a stoloniferous growth habit, which allows it to be propagated vegetatively. It will spread slowly under noncompetitive conditions and when conditions favor stolon growth. It has a very fibrous root system which can have a depth of 100 to 150 cm. It will produce a dense, fine-textured turf with dark green color throughout most of the growing season. From middle fall to early spring, the grass is dormant.

Establishment rate:

Plugs.—12-14 weeks with irrigation.

Sod.—1-2 weeks.

Sprigs.—not recommended.

Regions of adaptation: North to south, from the U.S. - Canada border to northern Mexico, east to west, from Missouri to California. The exact geographic region of adaptation is currently under investigation in a national test directed by the National Turfgrass Evaluation Program.

Blade:¹

Shape.—Long, slender.

Length.—12 cm.

Width.—1.6 mm.

Pubescence.—heavy, compared to other buffalograsses.

Leaf color:² Dark green, 137-A.

Mature plant height: 10 cm.

Above canopy stolons: Minimal compared to 'Prairie.'

Internode length:¹ 5 cm (internode 2).

Internode color:^{1,2} Yellow-green, 146-B.

Node width:¹ 2.8 mm (node 3).

Soil adaptation: Heavy soils—silty clay loam preferred, slightly acid to alkaline pH.

Female inflorescence: Present, heavy in early growing season. The inflorescence is a spike.

Male inflorescence: Absent.

¹ Measurements made on greenhouse grown plants.

² RHA Colour Chart Designations.

References

All references cited herein are hereby incorporated by reference in their entireties.

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What is claimed is:

1. A new and distinct perennial, female buffalograss cultivar, substantially as herein shown and described, distinguished by its dark green color, improved turfgrass quality, tolerance to low mowing, unique molecular marker pattern, hexaploid DNA content, vegetative propagation, and tolerance to heat, drought, cold, and low maintenance conditions.

* * * * *

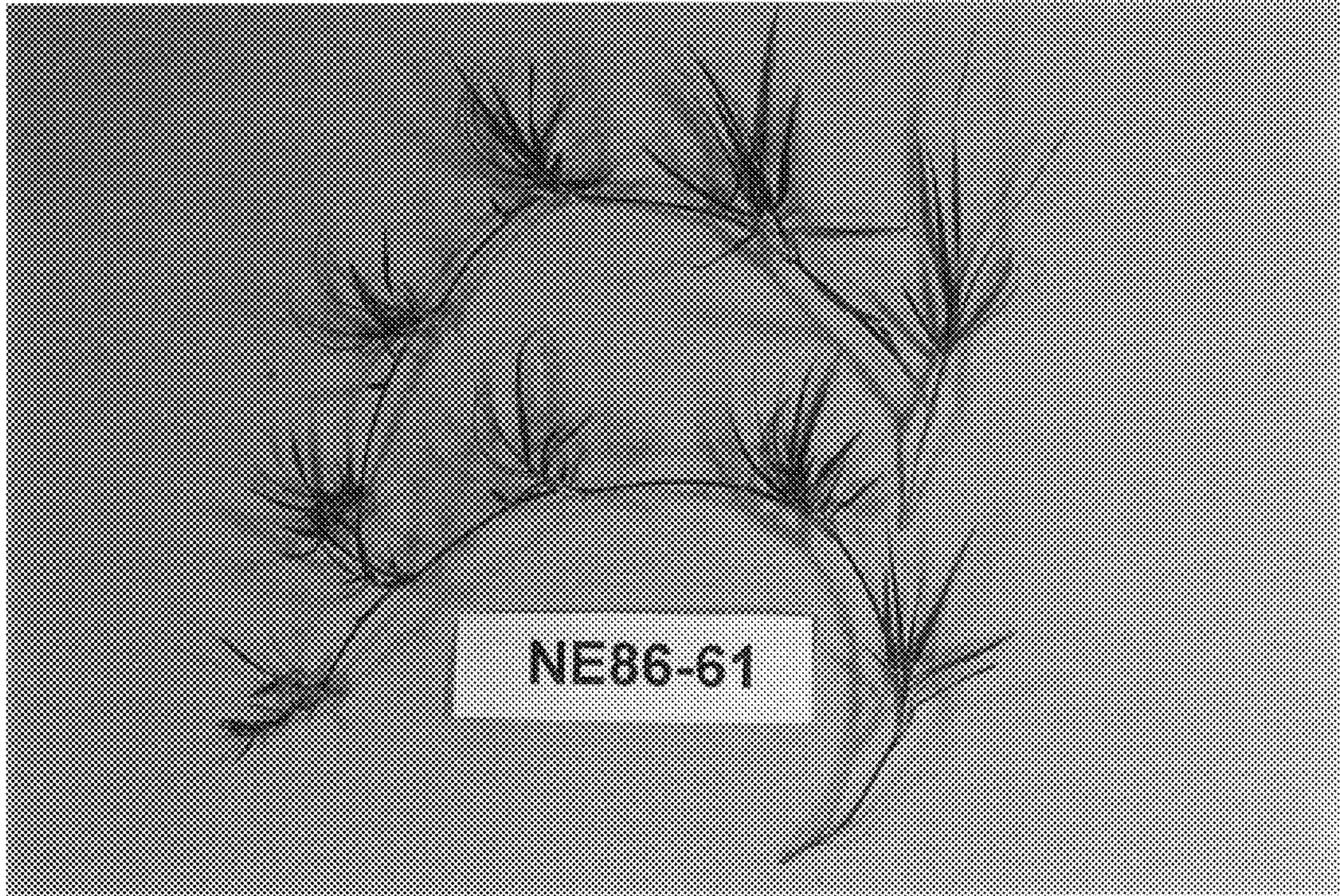


FIG. 1

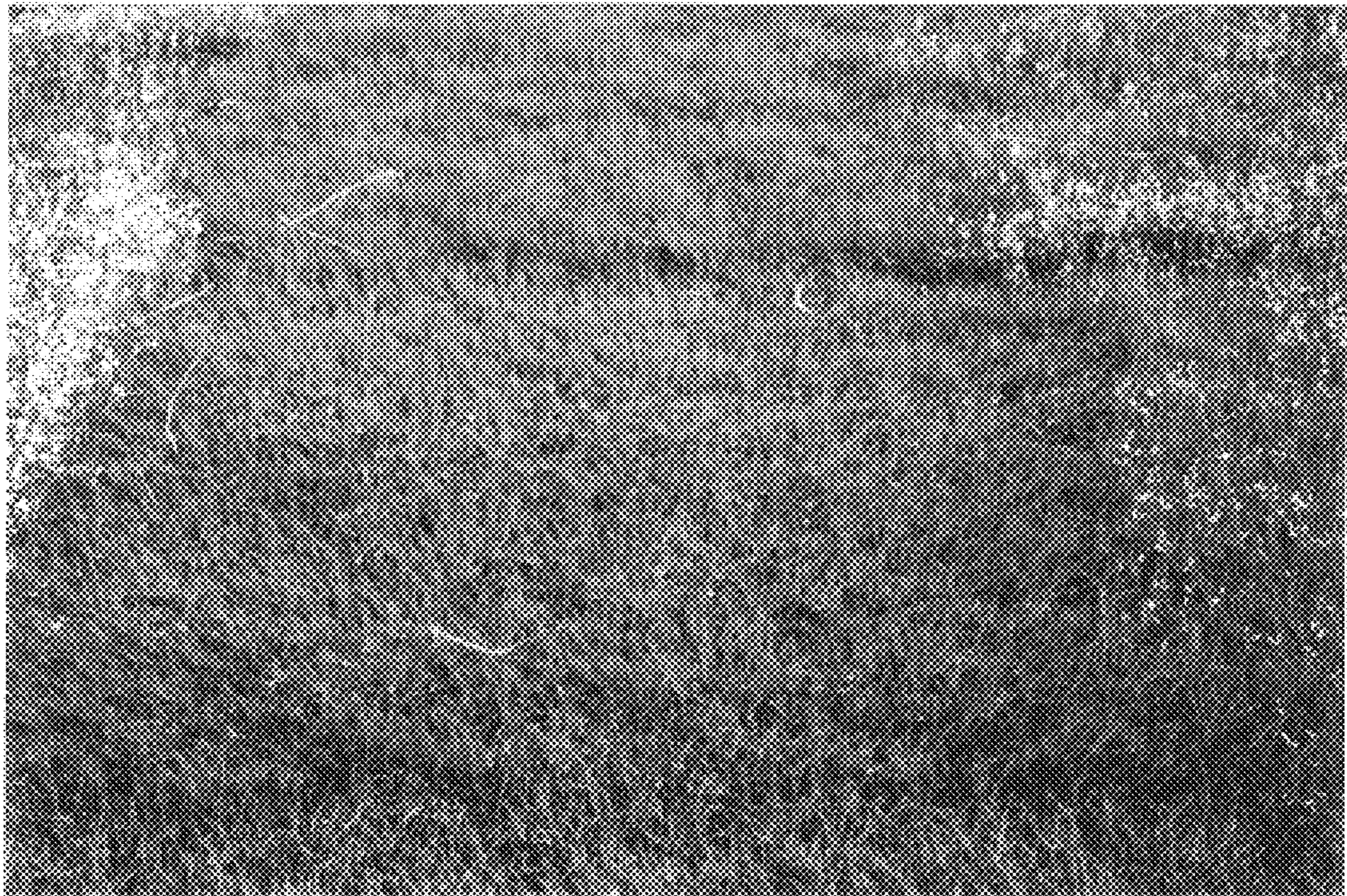


FIG. 2



FIG. 3

FIG. 4

