ALLEGANY, NEW YORK

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"indeterminate" is falsely day length, in maize

--Karl, JR

The "indeterminate" (*id*) mutation of maize may not be so indeterminate. Progress with it is impaired by false reports. The present study was an endeavor for clarification and to report the basic missing data.

Background

Recessive factors creating a dose of extra leaves on a maize plant have been reported since at least 1935 (early - Emerson et al., Cornell Agr Exp Sta Memoir 180:10, 1935). Extra leaves add extra days to the requirement for reproduction, to the extent that plants are commonly killed before one etiology can be distinguished from another. Additionally, accounts often lack information even about the wild-type plants. No comparisons can then be made to further the science. In this way, repeat publications have rendered themselves pointless.

The leaf addition in both *id* and delayed flowering are below the ear, whereas in a third event called short night leafy the addition is above ear. *id* has reproductive prohibitions unlike delayed flowering such as sterility or requiring longer reaching pollen shedding - and even prohibitively longer to reach silk protrusion.

Almost all maize scientists reveal a terminal absence of knowledge over the night length reaction of maize. The correlation of late maize flowering with the onset of autumnal night length is unfortunate (also expressly confessed by Langham, Genetics 25:93, 1940). It is projected onto *id. id* has been afflicted by the literature for 80 years (latest - Neuffer and Chang, MNL 63:62, 1989; Colasanti and Sundaresan, Maize Genetics Conference Abstracts 38,

1996; Maize GDB). Relentlessly, causality is claimed after being disproven (all on the same page, early example - Singleton, J Hered 37:63, 1946). Proof is neither offered nor required for the sweeping, untested conclusions. By some feat, the reports that reconciled the negligence are ignored.

It is almost impossible to navigate a definition of *id* from the literature. The basic data is absent. A person is forced to grow it themself to have an idea what it is. The author recently tested the material, with results startlingly unlike what anyone could venture to piece together.

Materials and methods

Two versions of the maize mutation "indeterminate" were grown in multiple environments. Seed from the bank envelope of the reference stock and from the bank envelope of the strongest version of the mutation (believed by JJ Colasanti, personal communication), Neuffer's 972, were grown in a long-night environment, in a short-night environment, in an environment with natural light, and in an environment with artificial light through the year of 2014. The fewest possible plants were grown. In the short-night environment of the New York field season, a (100 W) bulb was illuminated 3 ft above the plants for 3 h through the midsection of each night until tassel initiation, after which time leaf sheaths were removed to note ear placement (top ear initial). The artificial-light environment had no natural light, only two 1,000 W high-pressure sodium bulbs illuminated for 11 h each day for a 13 h nightlength. A long-night environment in the New York field season was made by covering plants each night with black sheet plastic from 7 PM to 8 AM for a 13 h nightlength. The stock for the natural light treatments was neither from the bank envelope nor did it include the reference stock; it was only the Neuffer stock, and it was a sib/OP increase from one plant from the bank envelope. That plant probably had 13 leaves and was one of three small plants out of a group of five raised from the bank envelope.

Parenthetical abbreviations: ART - artificial light, AE - above ear, LGHT - light effect, LN - long night, MUT - mutant, NL - nightlength, NAT - natural light, SYN - synergy between mutation and nightlength, SN - short night, WT - wild type

Data of wild type

Wild-type plants of both stocks had approximately 14 leaves under long night (3-4 AE) and 17 under short night (5-6 AE), a jump of 3 leaves, with pollen or silk in 2 mo 1 wk regardless of nightlength (reference stock 2.5 mo). In all cases of the wild type, the reference stock (14-15 LN, 17-20 SN, 3-5 NL) substantially tended toward more leaves than the Neuffer stock (12-15 LN, 15-17 SN, 2-3 NL).

Mutant data

The reference mutant had (estim.) 19 leaves under long night (14 WT + 5 MUT) and 24 in short night (14 WT + 5 MUT + 4 NL + 1 SYN; 6 AE) whereas the Neuffer mutant had 21 under long night (21-22 ART, 23 NAT; 13 WT + 8 MUT; 3-4 AE) and 27 in short night (30 NAT, 3 LGHT; 13 WT + 8 MUT + 3 NL + 3 SYN; always 6 AE). Time to tassel protrusion was basically the same regardless of mutant version - 5 mo under long night and 6 in short night - as were leaves above the ear, indicating that 3 is basically the minimum that these backgrounds could have, and 6 the maximum.

Clear summary

The data might be indicating (1) that one substantial dose of several leaves was created by the mutation, (2) a second substantial dose of several leaves was created by nightlength, (3) a third but marginal dose of a few leaves was created by a reaction between the mutation and nightlength, and (4) a fourth but marginal dose of a few leaves was created by natural light.

Leaf initiation and phase transition

When the data is applied to an equation by Russell and Stuber (Crop Sci 24 (3):507-510, 1984), it is identified that all of the mutant plants initiated their tassels before leaf collar 13 protruded from the whorl. This was ~40% of the way toward - more than two months from - flowering, revisiting the gaffe that tassel initiation is instantly followed by tassel protrusion. Even in the most extreme anecdotes of flowering in October, initiation probably occurred under early August nightlength, which is still generously over 13 h, if not even over 14 at the 5 ft-

candle level (June, 15 h; July, 14 h; August 13 h; September 12 h; Francis, Agron J 62:790-792, 1970). Ironically, full aerial rooting occurred to node 13, multiple feet in the air, on multiple mutant plants in the artificial-light environment.

Mutation-nightlength relationship

No promises of indeterminacy and long-night obligacy in maize have ever proven founded (two honest early reports - Bryan, MNL 12, 1938; Langham, MNL 12:10, 1938). Indeterminacy in *id* is disproven by flowering in all three of the early events, 1934-7, and obligacy, not to mention general nightlength relation, was disproven even in the report that coined the mutation.

As to nightlength, the difference in leaf number between long and short night was not due to the mutation, because the same change happened in wild type. In minor exception to that principle, nightlength may have had a small effect (up to 10% of leaf total, 3 leaves) on expression of the mutation.

The reports of the literature pretend that the nightlength effect and *id* are the same thing. Therefore, together, they look like an unbridled response (RF 70% more leaves, NF 110%). Without separating the two, one might conceivably see a multi-hundred percent increase (cf. Stevenson and Goodman, Crop Sci 12:864-868, 1972). In spite of all of the stupefying regard given to the *id* report of Galinat and Naylor (Am J Bot 38:38-47, 1951), they did not address background, which had preeminent bearing on their result. A reader might assume that the background was Singleton's, which had the very reactive population "Long Island Beauty." Galinat and Naylor reported that their wild type had a negative nightlength reaction at the same time that their mutant had a positive one as great as to stipulate long-night obligacy. The only thing that a reader could do with that was ignore it. Instead, Shaver (MNL 41:33, J Hered 58:273, 1967) alluded to it, positing that is was from greenhouse glass, and Colasanti (Colasanti and Sundaresan, MNL 66:31, 1992, cited with permission) excused it as allele version. What then remained in the data of the report was the proper, typical mutant flowering at 5 months. In the present investigation, the behavior of the mutant was the same regardless of environment. If long-night obligacy exists, it is harrowing to reproduce, let alone

with the weak reference allele as recorded by Galinat and Naylor. Long Island Beauty is long purged from the reference stock (M Sachs, personal communication), but there is nonetheless nightlength reaction in the present background.

This corroborates the superior strength of the Neuffer allele (60% increase in leaves) over the reference allele (30%). It would be fantastic to see it affected by the most reactive background of the subspecies, like late Tehua strains. The Neuffer allele is twice as potent as the reference allele, though the background was equally or slightly less reactive than that of the reference allele. In the Neuffer stock, the mutation-nightlength synergy and natural-light reaction were less marginal (20% inc. leaves) than in the reference stock (5%), which might suggest that the mutation is the independent variable of those reactions.

Greenhouse glass - artificial light

As to possible filtration of natural light by greenhouse glass, there was no qualitative difference in expression of the mutation (concerning leaf number) between artificial and natural light. The phenomenon of greenhouse glass/artificial light might concern not the qualitative presence of a wavelength of energy from the atmosphere (UV suggested), but shift in its preponderance, like the effect of blue-red ratio on internode elongation. Plants might have added a leaf or more from the absence of artificial light. Even mid-night bulbs ostensibly created the deficit.

Reproduction and phase transition

In this study, when the wild-type plants were under short night, they had normal reproduction. However, when in long night, they did not silk under artificial light. They did silk under natural light, but when pollinated, no seed developed.

In the mutant plants, pollen and aerial rooting were heavy under artificial light, whereas tassels were sterile and rooting nonexistent in plants under natural light. A reference mutant plant was permitted to grow for long enough for significant visible ear development, and showed a multi-centimeter cob with silk - just like in super leafy - making it seem that *id* might

silk normally if but given the time. Shaver (MNL) did isolate and enrich a strain in which $^{\sim}2/3$ plants did produce viable ear seed.