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Festigkeit von Zuckerrüben – Ursachen für Sortenunterschiede und die Auswirkungen auf Beschädigung und Lagerungsverluste

Festigkeit von Zuckerrüben – Ursachen für Sortenunterschiede und die Auswirkungen auf Beschädigung und Lagerungsverluste

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Abkürzungsverzeichnis

AiF	Arbeitsgemeinschaft industrieller Forschungsvereinigungen „Otto von Guericke“
AIR	alcohol insoluble residue
ANOVA	Analysis of variance
BMWi	Bundesministerium für Wirtschaft und Energie
°Cd	Gradtage
Cdist	Abstand (in mm) bis zum Bruch (Kompressionstest)
Cmax	Benötigte Kraft beim Bruch (Kompressionstest)
COBRI	Coordination of Beet Research International
DM	Drymatter (Trockenmasse)
E-Type	Zuckerrübensorten mit hohem Rübenertrag
EU	Europäische Union
FEI	Forschungskreis der Ernährungsindustrie
GEI	Genotyp-Umwelt-Interaktion
i.e.	id est
ICUMSA	International Commission for Uniform Methods of Sugar Analysis
IfZ	Institut für Zuckerrübenforschung
IIRB	International Institute of sugar beet research
IRBAB	Institut Royal Belge pour l'Amélioration de la Betterave
IRS	Stichting IRS
Marc/Mark	Heißwasser unlöslicher Rückstand
MS	mean square
N	Nitrogen
n.s.	not significant
N_{adv}	regional advised N fertilization
NBR	Nordic Beet Research
N_{min}	mineral N in soil in spring
N-Type	Zuckerrübensorten mit hohem Rübenertrag
NZ	Standort bei Hankensbüttel, Deutschland
NZ-Type	Zuckerrübensorten mit ausgeglichenem Verhältnis zwischen Rübenertrag und Zuckergehalt
p	Signifikanzwert
PCA	Principal Component Analysis

Pdist	Abstand (in mm) bis zum Bruch (Penetrationstest)
Pmax	Benötigte Kraft beim Bruch (Penetrationstest)
Pmean	Durchschnittliche Kraft von 0,5 mm nach Pdist bis zu einer Tiefe von 5 mm (Penetrationstest)
% Pol	polarimetrisch bestimmter Zuckergehalt
SBR	Syndrome Basse Richesse
SC	Sugar Content
SZ	Standort bei Eibelstadt, Deutschland
Z-Type	Zuckerrübensorten mit hohem Zuckergehalt

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1 Zuckerrübenlagerung

Der Zuckermarkt in der EU wurde ab 1968 durch die Zuckermarktordnung geregelt. Durch Produktionskontrolle mittels eines Quotensystems und durch Interventionspreise über dem Weltmarktniveau sollte der EU Zuckermarkt stabilisiert und der Lebensstandard der EU Zuckerrübenanbauern gesichert werden (Wimmer und Sauer 2020). Im Jahr 2006 wurde der europäische Zuckermarkt durch die Verringerung der Roh- und Weißzuckerpreise sowie Interventionspreise und durch die Abschaffung der Produktionsquote deutlich verändert (No.318/2006). Zur Anpassung an die verringerten Preise wurden zahlreiche Zuckerfabriken geschlossen, um Fixkosten durch längere Verarbeitungskampagnen der verbleibenden Fabriken zu senken.

In Deutschland wird eine Kampagnelänge von 120 Tagen angestrebt. Die Zuckerrübenernte beginnt meist Mitte September, endet Ende November und ist somit kürzer als die angestrebte Kampagnelänge. Bei früherem Erntebeginn ist das Ertragspotenzial noch nicht voll ausgeschöpft, während im Spätherbst schlechte Witterungsbedingungen die Ernte begrenzen und die Aussaat einer Nachfrucht, meistens Winterweizen, erschwert wird. Hohe Bodenfeuchte bei der maschinellen Ernte im Spätherbst kann zu langfristiger Verdichtung des Bodens führen (Håkansson und Reeder 1994). Daher muss ein Teil der Zuckerrüben zwischen Ernte und Verarbeitung in Feldmieten gelagert werden.

Während der Lagerung entstehen Lagerungsverluste, die insbesondere durch die Temperatur und die Lagerungsdauer beeinflusst werden. Eine Lagerung bis mindestens Mitte Januar entspricht einer Verlängerung der Lagerungsperiode auf mindestens 60 Tage oder einer Verdoppelung der bisherigen Lagerungsdauer (Huijbregts et al. 2013). Durch die verlängerte Lagerungsperiode erhöht sich die Menge an gelagerten Rüben, wodurch die Lagerungsverluste für die gesamte Wertschöpfungskette steigen. Dabei lassen sich zwei Kategorien unterscheiden: Einerseits entstehen direkte Ertragsverluste durch Zuckerabbau (Zuckerverluste), andererseits reichern sich während der Lagerung Nichtzuckerstoffe an (Martin et al. 2001), die die Verarbeitung beeinträchtigen. Der direkte Zuckerabbau verursacht finanzielle Verluste für Landwirte und Verarbeiter. Darüber hinaus werden für eine effiziente Verarbeitung von Zuckerrüben ein hoher Zuckergehalt und geringe Anteile von Nichtzuckerstoffen benötigt, da Nichtzuckerstoffe zu Problemen bei der Verarbeitung und zu höheren Verarbeitungskosten führen (van der Poel et al. 1998). Die Lagerungsverluste und Schwierigkeiten bei der Verarbeitung nach langer Lagerung stehen somit im Widerspruch zur Effizienzsteigerung durch verlängerte Verarbeitungskampagnen.

1.1 Ursachen und Einflussfaktoren der Lagerungsverluste

Zuckerrüben sind auch nach der Ernte noch lebende Organe und benötigen Energie zur Aufrechterhaltung ihrer Lebensprozesse. Diese Energie wird mobilisiert, indem zuckerrübeneigene Enzyme wie

Invertasen und Saccharose-Synthase die im Rübenkörper gespeicherte Saccharose in die Monosaccharide Glucose und Fructose spalten (Burba 1976; Klotz und Finger 2004), dabei hat die Saccharose-Synthase die größere Bedeutung. Im Anschluss werden die Monosaccharide in der Glykolyse veratmet. Wenn mehr Saccharose gespalten wird, als durch die Respiration zu CO_2 oxidiert, reichern sich Glucose und Fructose an. Zusammen werden sie als Invertzucker bezeichnet, welcher als Qualitätsparameter für die Verarbeitung von Zuckerrüben angesehen wird (Klotz et al. 2006; Vermeulen 2015). Invertzucker wirkt sich negativ auf die Verarbeitung aus, da dieser die Alkalität der Säfte verringert und zu Farbbildung führt (van der Poel et al., 1998). Ein erhöhter Invertzuckergehalt verringert die Verarbeitungsgeschwindigkeit, erhöht den Einsatz an Prozesshilfsmitteln und führt so zu einer schlechteren Effizienz der Verarbeitung.

Die Veratmung von Saccharose während der Lagerung der Zuckerrüben kann nicht verhindert werden, jedoch können produktionstechnische Maßnahmen diese bis zu einem gewissen Grad reduzieren (Kenter et al. 2006; Klotz und Finger 2004). Maßgeblich werden die Stoffwechselprozesse der Zuckerrübe von der Lagerungstemperatur bestimmt (Burba 1976; Kenter et al. 2006). Optimale Lagerungstemperaturen liegen zwischen 1,5 und 5 °C, da in diesem Bereich die Enzymaktivität gering ist (Campbell und Klotz 2006), während bei höheren Temperaturen die Umsetzungsprozesse erheblich zunehmen. Die Lagerungstemperatur wird in Europa nur durch das Abdecken der Mieten mit Stroh oder Vlies geregelt (Huijbregts et al. 2013). Diese Maßnahme verringert jedoch nur kurzfristig das Risiko von Frosteinwirkung. Frost führt zu irreversibler Zellschädigung, und nach Auftauen kommt es zu hohen Lagerungsverlusten durch Zuckerabbau und Anreicherung von Nichtzuckerstoffen (Campbell und Klotz 2006; Kenter und Hoffmann 2006). Wenn die Rüben allerdings gefroren bleiben, sind die Lagerungsverluste sehr gering, da der Stoffwechsel fast zum Erliegen kommt. Dies wird in Anbaugebieten in den nördlichen USA ausgenutzt, da die Lufttemperaturen dort über einen längeren Zeitraum sehr niedrig sind und die Zuckerrüben mit Hilfe von Ventilationssystemen im gefrorenen Zustand gehalten werden können (Backer et al. 1979).

In Deutschland ist das Risiko für Schäden durch Hitze während der Lagerung höher als das von Frost. Gerade die Abdeckung der Mieten kann bei hohen Umgebungstemperaturen die Temperatur der Miete deutlich erhöhen. Hohe Lagerungstemperaturen führen zu deutlich steigenden Lagerungsverlusten (Kenter und Hoffmann 2006; Kenter und Hoffmann 2009b). Somit ist im Gegensatz zu den kontrollierten Lagerungsbedingungen bei Äpfeln oder Kartoffeln die Temperatur in der Miete von den Witterungsbedingungen am Standort abhängig.

Um die Variabilität der Temperatur als Einflussfaktor auszuschließen, wurden die Lagerungsversuche in dieser Arbeit unter kontrollierten Bedingungen durchgeführt. Die Lagerungstemperatur wurde dabei an die durchschnittliche Lufttemperatur während der Lagerungsperiode in Deutschland angepasst.

Ein weiterer Faktor, der den Abbau von Zucker verstärkt, ist die Beschädigung der Rübe. Während der Ernte und des Transports sind Zuckerrüben unterschiedlichen mechanischen Beanspruchungen ausgesetzt, welche zu Beschädigungen des Rübenkörpers führen (Steensen 2002). Die besonders zu Beginn der Lagerung intensiv stattfindenden Wundheilungsprozesse verringern das Austreten von Zellsaft, wodurch das Risiko von mikrobiellen Infektionen sinkt (Cole 1977; Wyse 1978). Jedoch benötigt dieser Prozess Energie aus dem Kohlenhydratstoffwechsel und führt so zu Zuckerabbau und Lagerungsverlusten (Ibrahim 2001). Beschädigungen bilden zudem die Eintrittspforte für pathogene Mikroorganismen, die im Boden ubiquitär vorkommen (Liebe und Varrelmann 2016; Mumford und Wyse 1976). Mikrobieller Befall, begleitet von Fäulnis- und Schimmelbildung, führt zusätzlich zum Stoffwechsel der Zuckerrübe zur Umsetzung von Zucker und erhöht dadurch die Lagerungsverluste deutlich (Klotz und Finger 2004). Liebe und Varrelmann (2016) zeigten, dass das Mikroorganismenspektrum in unterschiedlichen Umwelten ähnlich war und zum Großteil aus Wundpathogenen bestand. Diese können jedoch nicht ohne weiteres intakte Zellwände durchdringen und sind somit auf vorherige Beschädigung angewiesen.

Den größten Einfluss auf die Bildung von Lagerungsfäulen hatte in der Studie von Liebe und Varrelmann (2016) die Umwelt. Es gibt viele umweltabhängige Einflussfaktoren, wie Boden, Witterungsbedingungen, agronomische Maßnahmen und Beschädigung durch unterschiedliche Roder und Rodereinstellungen, die die Intensität der Beschädigung und des Befalls mit Lagerungspathogenen bestimmen können. Das Auftreten von Lagerungsfäulen wurde jedoch häufig als zufällig beschrieben, da ebenfalls gesunde Zuckerrüben deutliche Symptome entwickeln können und bereits bei der Ernte ohne äußere Symptome befallen sein können (Campbell und Klotz 2007; Christ et al. 2011).

Auch die Sorte hat bei Zuckerrüben einen signifikanten Einfluss auf die Intensität des Befalls mit Lagerungsfäule und somit auch auf die Lagerungsverluste (Campbell und Klotz 2007; Liebe und Varrelmann 2016; Schnepel und Hoffmann 2016; van Swaaij und Huijbregts 2010). Dabei hat sich gezeigt, dass der Invertzuckergehalt nach der Lagerung mit den Zuckerverlusten und dem Fäulnisbefall korreliert (Kenter und Hoffmann 2009b; Liebe und Varrelmann 2016; Schnepel und Hoffmann 2016). Allerdings war es in offiziellen Sortenversuchen bislang nicht möglich, genotypische Unterschiede in den Lagerungsverlusten festzustellen (Kenter und Ladewig 2020). Unterschiede zwischen Sorten treten besonders ausgeprägt auf, wenn die Lagerungsdauer lang ist, die Temperaturen höher und der Pathogenbefall dadurch sehr hoch ist. Da die Pathogene auf Beschädigungen als Eintrittspforte angewiesen sind, ist die Beschädigung bei der Ernte ein weiterer entscheidenden Einfluss auf die Lagerungsverluste.

1.2 Beschädigung von Zuckerrüben

Das Ausmaß der Beschädigung während der Ernte hängt bei Zuckerrüben maßgeblich von den Rodereinstellungen ab (Hoffmann et al. 2018). Hohe mechanische Kräfte bei der Ernte führen einerseits zu Verlusten von Wurzelstücken, insbesondere der Wurzelspitze, aber auch zu bislang weniger beachteten Abschürfungen und Quetschungen (Wiltshire und Cobb 2000). Die Rodereinstellung wird maßgeblich durch die Umweltbedingungen bei der Ernte bestimmt. Unterschiedliche Böden und

Witterungsbedingungen erfordern angepasste Rodereinstellungen, um den Erdanhang gering zu halten, ohne das Niveau der Beschädigung deutlich zu erhöhen (Hoffmann et al. 2018).

Neben der Rodereinstellung wird das Ausmaß der Beschädigung auch von der Empfindlichkeit der Rübe bestimmt. Dabei gibt es genotypische Unterschiede, wie Untersuchungen mit kontrollierter Beschädigung von Rüben durch eine Reinigungstrommel zeigten (Hoffmann und Schnepel 2016; van Swaaij et al. 2003). Diese Unterschiede wirken sich auch in der Praxis aus, wie Hoffmann (2018) anhand von Daten aus Praxismieten in Deutschland bestätigte.

Parameter, die die Empfindlichkeit von Zuckerrübenwurzeln gegenüber mechanischer Beschädigung beeinflussen, sind bislang kaum bekannt. Für andere Kulturpflanzen wurden Turgor, Gewebestärke und das Verhältnis zwischen Zellwand und Zellvolumen als wichtige Größen identifiziert (Wiltshire und Cobb 2000). Bei Zuckerrüben scheint für die genotypischen Unterschiede in der Beschädigungsempfindlichkeit der heißwasserunlösliche Zellwandgehalt (Markgehalt) eine Rolle zu spielen (Hoffmann und Schnepel 2016; Smed 1998). Smed (1998) wies außerdem eine höhere Beschädigungsempfindlichkeit bei geringerer Festigkeit des Rübengewebes nach. Somit ist zu vermuten, dass Unterschiede in der Beschädigungsempfindlichkeit von Zuckerrüben mit den Textureigenschaften des Rübengewebes in Verbindung stehen.

2 Festigkeit

2.1 Festigkeit von Obst und Gemüse

Bei Obst und Gemüse ist die Texturmessung eine Standardmethode zur Bestimmung der Qualität und wird häufig ergänzend zu sensorischen Prüfpanels verwendet (Harker et al. 2002). Sie wird an frischem, gelagertem oder verarbeitetem Obst und Gemüse durchgeführt. Der Fokus liegt dabei meist auf der Bestimmung des Mundgefühls beim Verzehr, da dies ein wichtiges Kriterium für die Akzeptanz des Kunden ist. Die verwendete Methode für die Texturbestimmung hängt von dem Produkt und der zu bestimmenden Eigenschaft ab. Bei direkt verzehrtem Obst und Gemüse werden häufig Penetrations- oder Kompressionstests verwendet (Abbott 2004). Der in dieser Arbeit verwendete Begriff Festigkeit bezieht sich auf die drei durch Penetrations- und Kompressionstests gemessenen Parameter: Penetrationswiderstand, Gewebefestigkeit und Druckfestigkeit. Dahingegen beschreibt die Textur die Gesamtheit der mechanischen Eigenschaften wie Kraft und Elastizität.

Für Obst und Gemüse mit langen Lagerungsperioden vor dem Verzehr ist die Textur nach der Lagerung häufig wichtiger als die Textur nach der Ernte. Äpfel zeigten Sortenunterschiede in der Festigkeit nach der Ernte sowie im Erweichen während der Lagerung (Ahmadi-Afzadi et al. 2013). Dabei wurde festgestellt, dass Sorten mit einer hohen Festigkeit und geringem Erweichen während der Lagerung einen geringeren Pathogenbefall aufwiesen. Auch für Kartoffeln konnte festgestellt werden, dass sich die Sorten in ihrer Festigkeit unterschieden (Koch et al. 2019) und dass Genotypen mit festerem Gewebe eine geringere Anfälligkeit gegenüber Druckstellen hatten als weiche Genotypen (Castleberry und Jayanty 2017).

Die Textureigenschaften werden hauptsächlich durch den Turgor, die Zellgröße und die Zellwandzusammensetzung, einschließlich der pektinreichen Mittellamelle, bestimmt (Zdunek et al. 2014; Li et al. 2013). Die Zellwand bestehend aus Pektin, Hemicellulose, Cellulose und Lignin, kann sich während der Lagerung in ihrer Zusammensetzung verändern und damit die Festigkeit beeinflussen (Herppich et al. 2006). Des Weiteren stellte McGarry (1993) fest, dass sich die Wasserverfügbarkeit auf die Zellgröße und auf die Textureigenschaften von Möhren auswirkte und ein höherer Turgor zu einer höheren Beschädigungsempfindlichkeit führte. Eine regelmäßige Beregnung von Äpfeln führte unter normalen Wachstumsbedingungen ebenfalls zu einer Verringerung der Fruchtfleischfestigkeit (Opara et al. 1997). Auch bei Kartoffeln war die Festigkeit bei Knollen mit großen Zellen geringer als bei kleineren Zellen (Zdunek und Umeda 2005), zudem war die Beschädigungsempfindlichkeit bei Kartoffelsorten mit großen Zellen erhöht (Hudson 1975).

2.2 Stand des Wissens zur Festigkeit von Zuckerrüben

Während Festigkeit und Elastizität für Obst und Gemüse gut untersucht sind und verschiedene Methoden als Referenz etabliert wurden (Abbott 1999, 2004), gibt es für Zuckerrüben bisher keine umfangreichen Untersuchungen dazu. Die mechanischen Eigenschaften von Zuckerrüben wurden insbesondere in den 1970ern studiert (Drath 1976; Drath et al. 1984; Vukov 1972, 1975, 1977; Vukov und Pátkai 1978). Der Fokus dieser Arbeiten lag dabei auf Elastizität und Schneidwiderstand bei der Verarbeitung der Zuckerrüben in der Zuckerfabrik. Die Gewebestruktur der Zuckerrübe hat eine Bedeutung für die gesamte Verarbeitung (Buttersack und Basler 1991) und kann an vielen Stellen Einfluss haben, so bei der Schneidarbeit, Extraktion oder Abpressung. Vukov (1972) fand sortenspezifische Unterschiede im Schneidwiderstand, jedoch keine in der Elastizität. In vielen dieser älteren Untersuchungen wurden selbstentwickelte, nicht standardisierte Messgeräte verwendet, während neuere Untersuchungen mit Texture-Analysern realisiert wurden. In den meisten Fällen werden dazu Penetrations- und Kompressionstests durchgeführt (Gemtos 1999; Gorzelany und Puchalski 2000; Nedomová et al. 2017; Senge und Hajinezhad 2009). Bei einem Großteil dieser Untersuchungen lag der Fokus auf der Veränderung der Textureigenschaften während der Lagerung. Die Auswirkung von unterschiedlichen Umwelten oder Sorten auf die Textureigenschaften ist hingegen weniger erforscht (Gorzelany und Puchalski 2000; van Swaaij et al. 2003). Dabei verwendeten diese Studien auch keine einheitlichen Methoden, sodass ein Vergleich der Ergebnisse nur sehr eingeschränkt möglich ist.

2.3 Mögliche Ursachen und Einflussfaktoren auf die Festigkeit

Die Festigkeit von Zuckerrüben wird wahrscheinlich auch maßgeblich durch die Zellstruktur bedingt. Der Aufbau der Zuckerrübenwurzel wird von kreisförmig angeordnetem Leitgewebe, den sogenannten Kambiumringen, charakterisiert (Artschwager 1926). Die Bildung der Kambiumringe wird bereits früh durch die Blattbildung ausgelöst (Zamski und Azenkot 1981). Während des Dickenwachstums der Zuckerrüben entfernen sich die Ringe voneinander durch Zellteilung und Zellwachstum. Dabei ist der Abstand zwischen den inneren Ringen größer und nimmt nach außen hin ab. Die Anzahl an Kambiumringen steht in einem positiven Zusammenhang zum Zuckergehalt von Zuckerrübensorten, da vermutlich die Entfernung zwischen Phloem und Speicherparenchym eine wichtige Rolle für die Zuckerkonzentration spielt (Milford 1976; Wyse 1979). Zudem haben Zuckerrübensorten mit einem höheren Zuckergehalt kleinere Zellen (Milford 1976) und dementsprechend einen höheren Zellwandgehalt (Hoffmann et al. 2005). Zuckerrüben scheinen sich in den Ursachen, die bei anderen Arten für Unterschiede in der Festigkeit verantwortlich sind, ebenfalls deutlich zu unterscheiden.

Dass Sorte, Umwelt und landwirtschaftliche Maßnahmen die Textureigenschaften von Zuckerrüben beeinflussen, stellten bereits Drath (1976), Drath et al. (1984) sowie Vukov (1972, 1977) fest. Bislang gibt es jedoch nur Vermutungen für die Ursachen von Sortenunterschieden in der Festigkeit bei Zuckerrüben. Da Zuckerrübensorten sich in ertragreiche und zuckerreiche Sorten aufgliedern, gibt es vermutlich zwischen diesen Sorten auch große Unterschiede in der Zellgröße. Demnach hätten ertragreiche Sorten größere Zellen und somit vermutlich eine geringere Festigkeit. Der von Hoffmann und Schnepel (2016) gezeigte Zusammenhang zwischen Beschädigungsempfindlichkeit und unlöslichen Zellwandbestandteilen (Markgehalt) deutet daraufhin, dass der Zellwandgehalt auch bei Zuckerrübensorten die Festigkeit beeinflusst. Zudem hat sich der Zellwandgehalt im Verlauf des Züchtungsprozesses unbeabsichtigt verringert, sodass dieser bei neuen Sorten geringer war als bei alten Sorten (Loel et al. 2014). Somit kann davon ausgegangen werden, dass sich aktuelle Zuckerrübensorten von älteren Sorten unterscheiden. Es ist bisher nicht untersucht, ob auch bei einem geringeren Zellwandgehalt aktueller Sorten noch Unterschiede zwischen den Sorten nachweisbar sind. In dieser Arbeit wird der Zellwandgehalt als alkoholunlösliche Bestandteile bestimmt (AIR), da dieser im Gegensatz zum heißwasserunlöslichen Markgehalt auch wasserlösliches Pektin enthält (Kenter und Hoffmann 2009a) und somit für die Analyse der Zellwandzusammensetzung benötigt wurde.

Der Zellwandgehalt (Markgehalt) von Zuckerrüben wird neben der Sorte hauptsächlich von der Umwelt bestimmt (Hoffmann et al. 2005). Zudem bestimmt die Umwelt, insbesondere durch das Wasserangebot, die Ertragsbildung und hat damit Einfluss auf den Zuckergehalt und die Zellgröße. So wiesen bereits Drath et al. (1984) nach, dass das Wasserangebot die Textureigenschaften beeinflusst. Auf landwirtschaftlichen Flächen kann die Wasserverfügbarkeit durch eine Beregnung verändert werden.

Auch weitere agronomische Faktoren, wie die Stickstoffdüngung, können die Zellgröße als mögliche Ursache der Festigkeit beeinflussen. So zeigten Milford und Watson (1971), dass die Zellen mit steigender Stickstoffverfügbarkeit größer werden. Dies führte bereits zu der Vermutung, dass Stickstoff die Empfindlichkeit von Zuckerrüben gegenüber Quetschungen steigern kann (Wiltshire und

Cobb 2000). Erste Untersuchungen zeigten bereits, dass eine deutlich überhöhte Stickstoffdüngung die Textureigenschaften verringerte (Drath et al. 1984).

Unterschiede zwischen Sorten im Zucker- und Markgehalt sowie vermutlich in der Zellgröße lassen annehmen, dass es auch Unterschiede in der Festigkeit der Rübe gibt. Die Festigkeit könnte als erstes Merkmal für physikalische/mechanische Eigenschaften in die Sortenzulassung integriert werden, wenn sich zeigt, dass es genotypische Unterschiede gibt und diese eine Auswirkung auf die Beschädigungsempfindlichkeit und Lagerfähigkeit haben. Zugelassen werden in Deutschland nur Sorten, die im Mittel über unterschiedliche Umwelten einen höheren Ertrag, bessere Qualität oder einen bestimmten landeskulturellen Wert, z.B. höhere Resistenzen/Toleranzen, aufweisen als bereits zugelassene Sorten. Diese mittlere Leistung ist jedoch nur relevant, wenn die Sorten in unterschiedlichen Umwelten einheitlich reagieren. Wenn dies nicht der Fall ist, liegt eine Genotyp-Umwelt-Interaktion vor, die die Untersuchung und Züchtung einer gewünschten Eigenschaft erschweren würde (Annicchiarico 2002; Hoffmann et al. 2009). Die stärkste Form der Interaktion ist die Crossover-Interaktion. Dabei wechseln die Sorten die Rangfolge zwischen den Umwelten (Baker 1988). Für die Ertrags- und Qualitätsparameter von Zuckerrüben stellten Hoffmann et al. (2009) fest, dass die Genotyp-Umwelt-Interaktionen im Vergleich zu Getreide sehr niedrig waren und keine Crossover-Interaktionen auftraten. Ob die Festigkeit von Zuckerrübensorten ebenfalls umweltstabil ist und somit anhand weniger Umwelten erfasst werden kann, muss jedoch erst geprüft werden.

3 Ziel der Arbeit

Für verschiedene Obst- und Gemüsearten ist bekannt, dass sich die Festigkeit bei der Ernte auf die Beschädigung während Ernte und Transport und auf das Lagerungsverhalten auswirkt. Durch die mechanisch sehr intensive Zuckerrübenernte wirken hohe physikalische Kräfte auf den Rübenkörper. Daher werden die Zuckerrüben trotz angepasster Rodereinstellungen beschädigt. Es ist bekannt, dass es genetische Unterschiede in der Beschädigungsempfindlichkeit und in Lagerungsverlusten gibt. Jedoch sind Versuche zur Beschädigungsempfindlichkeit und Lagerfähigkeit sehr zeitaufwendig und teuer und somit in der Züchtung und Sortenzulassung wenig praktikabel. Um diese Merkmale züchterisch zu bearbeiten, fehlen bislang einfach zu messende Parameter. Daher ist Ziel dieser Arbeit zu untersuchen, ob die Festigkeit von Zuckerrüben ein zuverlässiges und einfach zu bestimmendes indirektes Selektionskriterium für die Lagerfähigkeit sein kann.

Da es keine standardisierte Methodenbeschreibung zur Messung der Festigkeit von Rüben gibt und bisherige Studien daher nur begrenzt vergleichbar sind, sollte zunächst eine Methode beschrieben werden, welche die Wiederholbarkeit und Vergleichbarkeit von Ergebnissen gewährleistet.

Mithilfe dieser Methode sollten folgende Aspekte untersucht werden, um ein größeres Verständnis über die Festigkeit von Zuckerrüben zu erlangen:

i. Welchen Einfluss haben Sorten und Umwelt auf die Festigkeit. Gibt es eine Genotyp Umweltinteraktion, die eine Messung und züchterische Bearbeitung erschwert?
ii. Welche physiologischen Ursachen sind für Unterschiede in der Festigkeit von Zuckerrüben verantwortlich?
iii. Welche Auswirkung hat die Festigkeit auf die Beschädigungsempfindlichkeit und Lagerfähigkeit von Zuckerrüben?
iv. Kann die Festigkeit durch landwirtschaftliche Maßnahmen beeinflusst werden?

4 Aufbau der Arbeit

Die Ergebnisse der Untersuchungen liegen in der vorliegenden Arbeit in Form von vier in wissenschaftlichen Journalen veröffentlichten Manuskripten vor.

Manuskript I

G. KLEUKER; HOFFMANN, C. M. (2019): Method development for the determination of textural properties of sugar beet roots. In: Sugar Ind. 144, 392–400. https://doi.org/10.36961/si23306.

Manuskript II

G. KLEUKER; HOFFMANN, C. M. (2021): Tissue strength of sugar beet root – genotypic variation and environmental impact. In: Crop Sci. 61, 2478–2488. https://doi.org/10.1002/csc2.20523.

Manuskript III

G. KLEUKER; HOFFMANN, C. M. (2022): Causes of different tissue strength, changes during storage and effect on the storability of sugar beet genotypes. In: Postharvest Biol. Technol. 183, 111744. https://doi.org/10.1016/j.postharvbio.2021.111744.

Manuskript IV

C. M. HOFFMANN; KLEUKER, G.; WAUTERS, A; ENGLISH, W; LEIJDEKKERS, M. (2022): Root tissue strength and storage losses of sugar beet varieties as affected by N application and irrigation. In: Sugar Ind. 147, 34-41. https://doi.org/10.36961/si28254

Manuskript I:

Method development for the determination of textural properties of sugar beet roots[1]

Methodenentwicklung zur Bestimmung der Textureigenschaften von Zuckerrüben

Gunnar Kleuker and Christa M. Hoffmann

Abstract

The harvest of sugar beet leads to root tip breakage and surface damage through mechanical impacts, which increase storage losses. For the determination of textural properties of sugar beet roots with a texture analyzer a reliable method description is missing. This study aimed to evaluate the impact of washing, soil tare, storage period from washing until measurement, sample distribution and number of roots on puncture and compression measurements. For this purpose, in 2017 comprehensive tests were conducted with sugar beet roots grown in a greenhouse. In a second step these tests were carried out with different *Beta* varieties from a field trial, and in addition, a flexural test was included. Results show that the storage period after washing and the sample distribution had an influence on the puncture and compression strength. It is suggested to wash the roots by hand before the measurement and to determine the strength no later than 48 h after washing. For reliable and comparable results a radial distribution of measurement points around the widest circumference of the root is recommended for the puncture test. The sample position of the compression test had an influence on the compressive strength and therefore, needs to be clearly defined. For the puncture and the compression test it was possible to achieve stable results with a small sample size, but with increasing heterogeneity of the plant stand a higher number of roots is required. The flexural test showed a high variability and is, therefore, not recommended for the analysis of sugar beet textural properties.

Keywords: Sugar beet root, Texture, Puncture, Compression, Bending, Flexural, Method description, Sample preparation, Sample size, Sample position, Texture analyzer

[1]G. KLEUKER; HOFFMANN, C. M. (2019): Method development for the determination of textural properties of sugar beet roots. In: Sugar Ind. 144, 392–400. https://doi.org/10.36961/si23306.

1. Introduction

Mechanical harvest and cleaning of sugar beet, followed by transport, often result in substantial root tip breakage and surface damage through mechanical impact (Gorzelany and Puchalski 2000; Van Swaaij et al. 2003). The extent of damages from topping or defoliation and root tip breakage highly depends on the harvester settings, and therefore, on the driver (Hoffmann et al. 2018a). In addition to the yield loss through damage, it has been shown that the sugar loss during beet storage is influenced to a large extent by the amount of damage (Hoffmann and Schnepel 2016). Furthermore, this and other observations suggest, that the damage susceptibility depends on the sugar beet variety (van Swaaij et al. 2003; Hoffmann et al. 2018b). This can probably be attributed to the stability of the root tissue. Mechanical properties of sugar beet were especially studied in 1970s and 1980s (Vukov 1972, 1975, 1977; Vukov and Pátkai 1978; Drath 1976; Drath et al. 1984), because soft sugar beet caused problems during slicing and pulp pressing in the sugar factories. Therefore, these authors measured the elasticity and the cutting resistance of the roots with different tools. They also studied the effect of different factors, such as variety, site and fertilizer rate on physical properties of sugar beet.

More recent studies on mechanical properties of sugar beet were published by Gemtos (1999), Gorzelany and Puchalski (2000), van Swaaij et al. (2003), Senge and Hajinezhad (2009) and Nedomová et al. (2017). In these studies different methods were used, in most cases puncture and compression tests with a texture analyzer. These approaches to describe textural properties are very common in the analysis of fruits, vegetables and processed foods (Sila et al. 2006; Nedomová et al. 2016). For sugar beet, a reliable method description, which provides recommendations for the handling of the roots before and during measurement, is still missing.

In particular, it is not clear, whether the soil adhering to the root after harvest (soil tare) has to be removed before measurement and to which extent the time period from washing until measurement can affect the results. It can be assumed that only textural properties of the root surface, such as the periderm strength, will be affected by a washing procedure, therefore, the puncture test was in focus here. Furthermore, it is not known to which extent a washing procedure can affect the sugar beet surface, so that the firmness is altered as compared to a root in the field.

A further problem can arise as a sugar beet root is not a homogenous body (Senge and Hajinezhad 2009). The internal tissue structure, mainly the density of the cambium rings, changes within the root (Artschwager 1926). Therefore, the position, from which a sample is taken, may determine the result. But it is neither known, if the sample position has an effect on the results nor, how many roots need to be measured to receive stable results.

When a large number of samples has to be tested for textural properties, it is not possible to do all measurements on the same day. Therefore, there could be a delay between the measurements of different plots. It has not been tested yet, how long roots can be stored before measurement without affecting the results.

In this study comprehensive tests were carried out to examine the effects of sample preparation, sample position and sample size on puncture and compression tests. In a second step these methods, and additionally flexural tests, were verified with roots from a field trial with different *Beta vulgaris* varieties. The given descriptions and recommendations for these methods will allow a uniform and comparable implementation in future studies.

2. Material and methods

2.1. Plant material

The sugar beet roots for the pre-test were taken from a pot experiment in the greenhouse in 2017. Three varieties were chosen, which differed in yield formation and represented extremes of the sugar beet types: Z-type (high sugar content), N-type (normal type), and E-type (high root yield). Exceptions were the roots for the washing test, which were from a local field with an N-type variety.

The adapted methods were then tested with roots from a field trial with six replicates (plots) in a randomized block design at two sites with loamy soil in 2017. It included two sugar beet varieties, one fodder beet and one beetroot (red table beet). The sugar beet varieties were identical with the Z- and E-type from the pot experiment. The trial sites were located 20 km north and south of Göttingen. The crop was grown according to Good Agronomical Practice and roots were harvested by hand in mid of October.

2.2. Pre-test

The pre-testing should provide insights about the procedure of a texture analysis of sugar beet roots. All roots were stored at 6 °C in a climate chamber. Before measurement the roots were warmed up to room temperature overnight to reduce possible temperature effects on mechanical properties of the tissue.

2.2.1. Washing test

To determine the effect of washing, the puncture test was performed with 10 sugar beet roots, first unwashed (with soil tare), and then again after washing by hand. The washed roots were stored again at room temperature, so that the root surface could dry off.

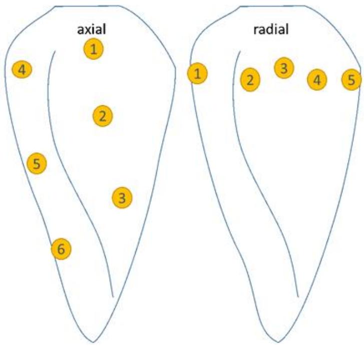
2.2.2. Storage period after washing

Along with the washing test, the influence of the storage period between washing and measurement was studied at 12 sugar beet roots of the Z- and E-type varieties (puncture test). Each root was measured immediately after washing (reference), and then again after 3 h, 6 h, 24 h, 48 h, 120 h and 168 h after washing with three measurements per root.

2.2.3. Distribution of measurement positions / sample position

To determine the impact of the distribution of the measurement points across the root, two puncture tests were conducted with 29 roots of the N-type variety. The 6 measurement points per root were either spread axially from the top to the bottom, or radially at the widest point around the root circumference (Fig. 1 A).

In case of the compression test different positions of the sample were tested using roots from the field trial. The cylinders were sampled from the outside to the centre of the root: Sample position 1 was at the outside near the periderm, sample position 3 at the central cylinder, and sample position 2 in between (Fig. 1 B).



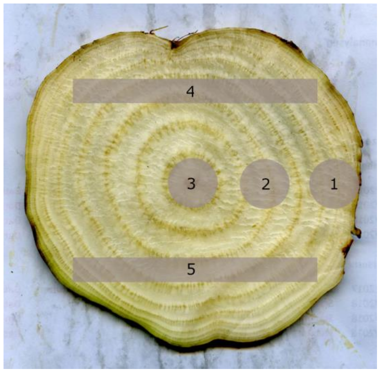


Fig. 1: (A) Axial and radial distribution of puncture measurement points across a sugar beet root. (B) Sample positions for the compression test (1 to 3) and sample cubes (4 and 5) for the flexural test.

2.3. Field trial

The aim of the field trial was to test the developed methods at a larger scale, to identify the minimum sample size and the feasibility of the method. The four varieties represent a broad range of *Beta* genotypes.

2.4. Texture analysis

Mechanical properties of the root were determined by puncture, compression, and flexural tests using a texture analyzer equipped with a 100 kg load cell (TA.XTplus100, Stable Micro Systems, Godalming, UK). To ensure comparability, the measured force of puncture and compression test F was converted to MPa taking the size of the sample or probe A into account (eq. (1)).

$$\sigma = \frac{F}{A} \tag{1}$$

The flexural strength was calculated according to DIN EN ISO 178, with the support span (l), width (w) and height (h) ((eq. (2)).

$$\sigma = \frac{3FL}{2wh^2} \tag{2}$$

2.4.1. Puncture test

Puncture testing was conducted with a 2 mm cylindrical probe (*P*/2) and a crosshead speed of 60 mm min^{-1}. The root was held perpendicular to the probe by hand. The puncture test was performed up to a penetration depth of 5 mm. The maximum force, which is needed to penetrate the periderm of the root, is the puncture resistance, while the average force from 0.5 mm after rupture until 5 mm represents the tissue firmness (Fig. 2). Root groove and crown were omitted

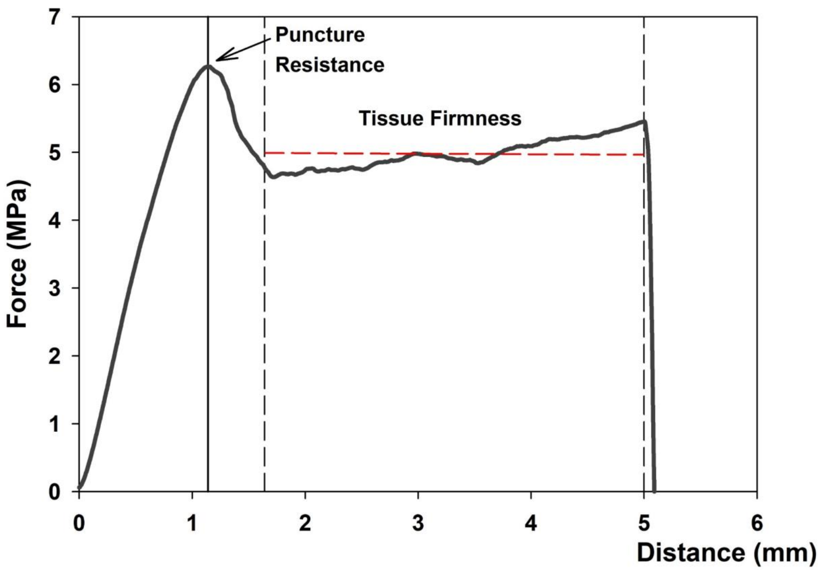


Fig. 2: Typical force-distance curve of a puncture measurement of a sugar beet root. Solid vertical line: puncture resistance; dashed horizontal line: calculated tissue firmness between dashed vertical lines.

for measurement. The measurement was repeated six times for each root. With five roots per plot and six replicates in the field a total of 180 measurements per variety were performed.

2.4.2. Compression test

The compression test was performed with three cylindrical samples per root. For the preparation of the samples a slice of at least 20 mm was cut out at the widest circumference of the root with a bread slicer (Raadvad). The samples were distributed horizontally across the slice (Fig. 1 B) and were cut with a cork borer (Ø: 18 mm) and trimmed to a height of 20 mm with a knife. The cylinders were compressed with a 75 mm compression platens (*P*/75) until rupture with a crosshead speed of 60 mm min^{-1}. The maximum pressure at rupture was measured as compressive strength. The compressive strength of the three samples per root was averaged to estimate the mechanical response of the whole root. Three samples of five roots in six field replicates resulted in 90 compression measurements per variety.

2.4.3. Flexural test

The cuboids for the flexural test (bending test) were sampled next to the compression cylinders in the same slice (Fig. 1 B). They were cut with a knife to a length of approximately 10 cm, 1 cm height and 2 cm width. The test was performed on a three-point bend rig (HDP/3PB), measuring the force at rupture. The support span was 23 mm. The flexural test was performed at two cuboids per root, with five roots in six field replicates these were 60 measurements per variety.

2.5. Statistics

Statistical analysis was carried out with the program SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The data were checked for normal distribution and homogeneity of variance and subsequently analyzed with the PROC Mixed function followed by *Tukey* test (Kozak and Piepho 2018). The calculation is based on the plot means. Pairwise comparison was computed with a paired *T*-test and *Dunnett's* test was used for test against a control group. Significant effects are indicated with * for $p \leq 0.05$, ** for $p \leq 0.01$ and *** for $p \leq 0.001$. Not significant was abbreviated to "n.s.".
The box-plots represent the mean value of the measured roots, where 50% of the values are included in the box. The whiskers define the upper and lower decile, 10% of the values are higher or rather lower than the end of the whiskers. The 5^{th} and 95^{th} percentiles of the values are shown as symbols. Median and mean are given as line and dashed line.

3. Results and discussion

3.1. Washing test

The mean puncture resistance of the sugar beet roots was 5.8 MPa before, and after washing as well (Fig. 3). The tissue firmness increased only slightly, but not significantly, from 4.57 MPa before to 4.68 MPa after washing. Thus, puncture resistance and tissue firmness were not influenced by the washing procedure or the adhering soil.
Washing may affect the measurement, since water changes periderm properties such as firmness, while the measurements of unwashed roots could be highly variable because of soil covering the root surface. Therefore, periderm stability (mean value) and the variability of the measurements (standard

deviation) were considered. Since no significant effects of washing were found, neither on puncture resistance nor on tissue firmness, it can be expected that washing did not alter periderm stability and that the texture analysis after washing represents the mechanical characteristics of roots from the field.

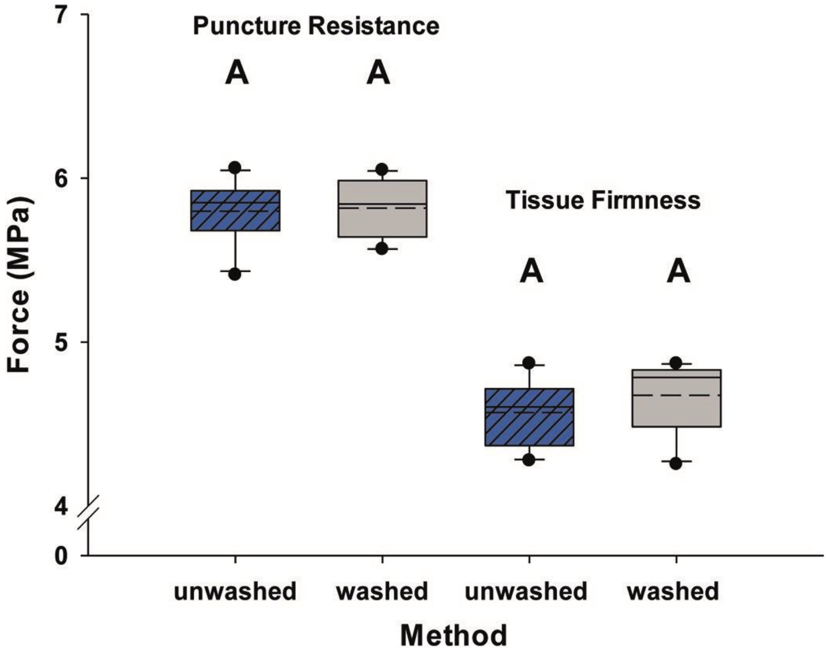


Fig. 3: Effect of washing on puncture resistance and tissue firmness of sugar beet roots; boxplots include 10 sugar beet roots with 3 measurements. T-test for each parameter separately. Same letters indicate no significant differences in means between treatments.

The test should also examine the possible effect of soil tare on the measurement. However, the soil adhering to the roots was very low in the present study (approximately 3%), and therefore, the impact should be negligible. In autumn often higher soil tare occurs, which may then strongly affect the measurement. Therefore, it is recommended to wash the roots before testing in order to obtain reproducible and reliable results. This applies particularly for roots with high soil tare.

Hand-held penetrometers are available, which offer the option to determine the penetration resistance of sugar beet during the growing period in the field (before harvest). As mentioned above, soil covering the root surface could disturb the measurement. A further problem could arise as the penetrometer is not fixed, so that even slight hand movements will possibly affect the measurement, in particular with regard to the variability of the data. It has to be tested whether hand-held penetrometer measurements can provide a reliable indication of the firmness of sugar beet roots from field measurements.

3.2. Storage period after washing

Figure 4 shows the effect of time period after washing on puncture resistance and tissue firmness for two sugar beet varieties. In case of puncture resistance no significant difference between the reference measurement (0 h after washing) and subsequent measurements was found. The tissue firmness decreased from 5.78 MPa to 5.16 MPa for the sugar beet Z-type, and from 4.91 MPa to 4.54 MPa for the E-type. Therefore, a significant difference in the tissue firmness between the reference (0 h) and 48 h after washing was found. However, the difference between the two varieties was significant at any time. The test of the influence of the storage period was regarded as necessary since storage can modify the textural properties of sugar beet roots (Nedomová 2017). The longer the time between washing and measurement, the more the tissue properties can alter. Results demonstrate, that a storage time of

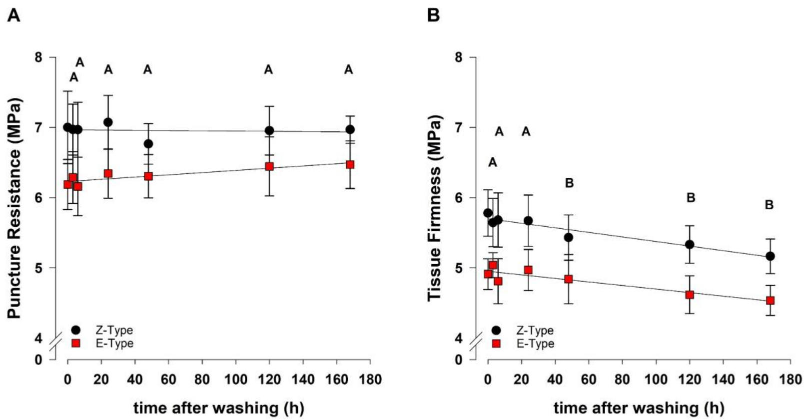


Fig. 4: Effect of time after washing on (A) puncture resistance and (B) tissue firmness of two sugar beet varieties; mean of 12 roots with 3 measurements. Vertical bars indicate standard deviation. *Dunnett's* test against reference measurement (0 h after washing). Different letters indicate significant differences to reference measurement.

seven days after washing did not affect the puncture resistance of the sugar beet varieties in comparison to the first measurement, while the tissue firmness decreased gradually. However, significant differences in tissue firmness to the first measurement did not occur within 48 h after washing. Therefore, in the best case texture measurements should be carried out as soon as the roots have dried and warmed up to room temperature. There seems to arise no change in varietal differences, even when the measurement is carried out after more than 48 h. That provides some more time for the handling in trials with many samples.

3.3. Distribution of measurement points / sample position

The radial measurements resulted in a significantly higher puncture resistance (5.64 MPa) than the axial measurements (5.28 MPa), while tissue firmness was not affected by the position of measurements (Fig. 5). The standard deviation of the measurements was similar.

As none of the two distributions tested (radial / axial) could prove a more stable or reasonable result, the practicability of the measurement was considered to determine the further procedure. The radial measurement was easier to handle because of the shape of the sugar beet root. In addition, the radial distribution around the broadest part of the root is better defined and narrows the space for possible measurements, so that the reproducibility and the repeatability (variability between different laboratories) is expected to be higher. Furthermore, as this tissue is rather homogenous, this may reduce the number of repetition measurements per root. For these reasons, the radial distribution is the preferred and recommended method.

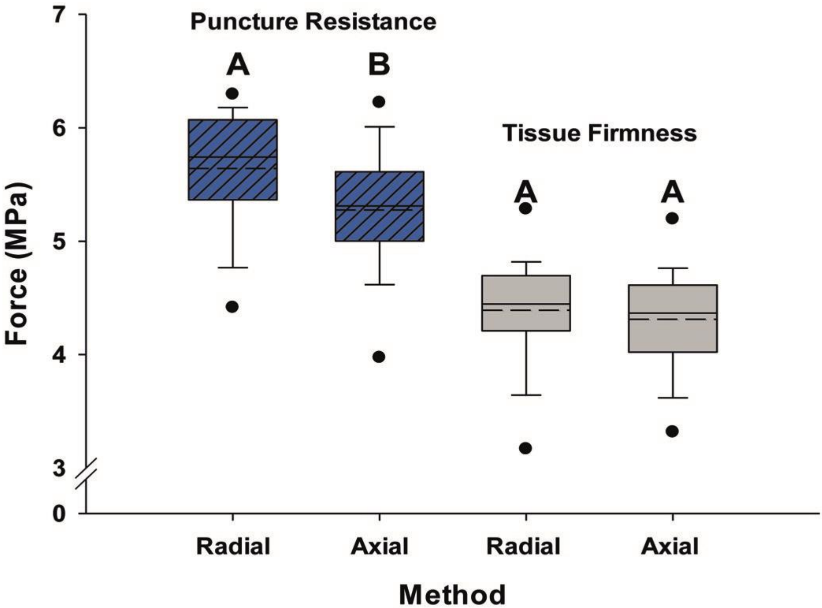


Fig. 5: Effect of radial and axial measurement point distribution on puncture resistance and tissue firmness of sugar beet roots; 29 sugar beet roots with 6 measurements. T-test for each parameter separately, different letters indicate significant differences in means between radial and axial measurements.

The compressive strength differed with the sample position (Fig. 6). For all tested varieties the strength significantly decreased from sample position 1 to 3, thereby decreasing from the outside to the centre of the root.

For the compression test, the decreasing compressive strength from the outside to the centre of the root underline that the sample position has to be well-defined within the cross section. The measured compressive strength of a sample position, and of the varieties as well, was in line with the density of cambial rings, which also decrease from the outside to the centre (Hoffmann 2010). Nedomová et al. (2017) measured the compressive strength only in the central region of the root (sample position 3) and obtained with 2.08 MPa a similar result as

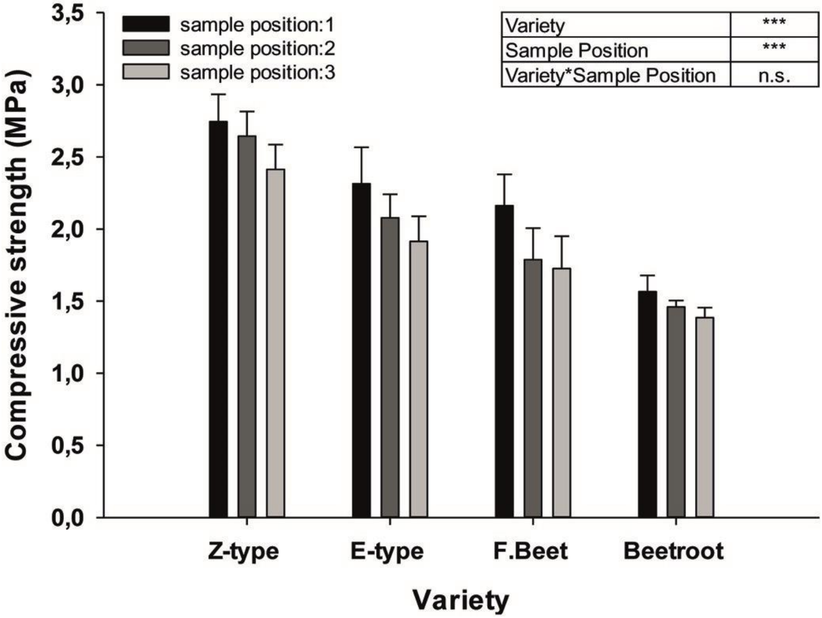


Fig. 6: Effect of sample position on compressive strength of sugar beet roots; mean of 30 roots. Vertical bars indicate standard deviation. * indicate significant differences at $p < 0.001$, n.s. = not significant.**

the E-type sugar beet. Gemtos (1999) also found differences between sample positions, but he found a higher compressive strength in the central part of the root. His explanation was that in the inner part the parenchyma cells are more frequent, suggesting that parenchymal tissue could exhibit a higher resistance compared to cambial rings with its high proportion of vascular tissue. However, that explanation could not be confirmed with present work.

3.4. Sample size / number of roots for puncture and compression test

The number of measurements resulted from the number of roots per plot, measurements per root, and replicates of the variety (treatment) in the field trial. Figure 7 shows the impact of the number of roots measured on the compressive strength and tissue firmness exemplary for the Z-type and the beetroot in the field trial. For that purpose, the mean and standard deviation were calculated, increasing the sample size stepwise, by adding the measured values per root in a randomized order. The graphs visualize that all mean values, except for the compressive strength from one root only, were within the same standard deviation from the mean value for 30 tested roots. Differences between the varieties were constant, irrespective of the number of roots tested.

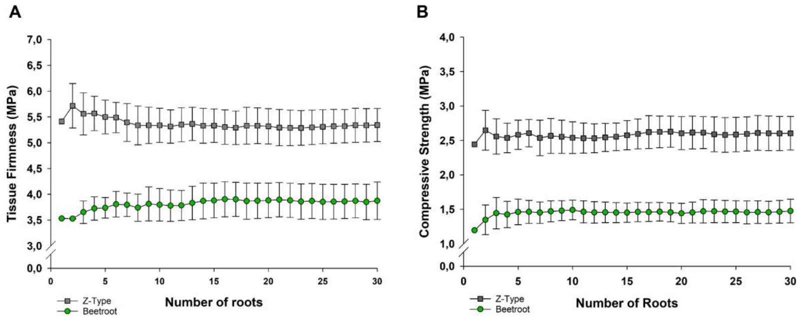


Fig. 7: Impact of the reduction of the number of roots on (A) tissue firmness (puncture test) and (B) compressive strength (compression test) for sugar beet and beetroot;

mean of 6 measurements per root for the puncture test and mean of 3 sample positions per root for compression test. Vertical bars indicate standard deviation.

A reduction of the number of measurements in the puncture test can result from the number of roots and from the measurements per root. Figure 8 shows the reduction of measurements by reducing the number of roots and measurements per root. Even the reduction to one root per field replicate (plot) with one measurement only did not lead to a significant interaction between variety and method. Differences between varieties could thus be identified well, irrespective of the number of measurements per plot.

For the determination of textural properties in sugar beet trials with many different plots, the number of measurements per plot needs to be reduced to the necessary minimum to save time and costs. The minimum should be defined as to give reliable and stable results, which means that the mean value should not change when fewer measurements are included, and the standard deviation should be low.

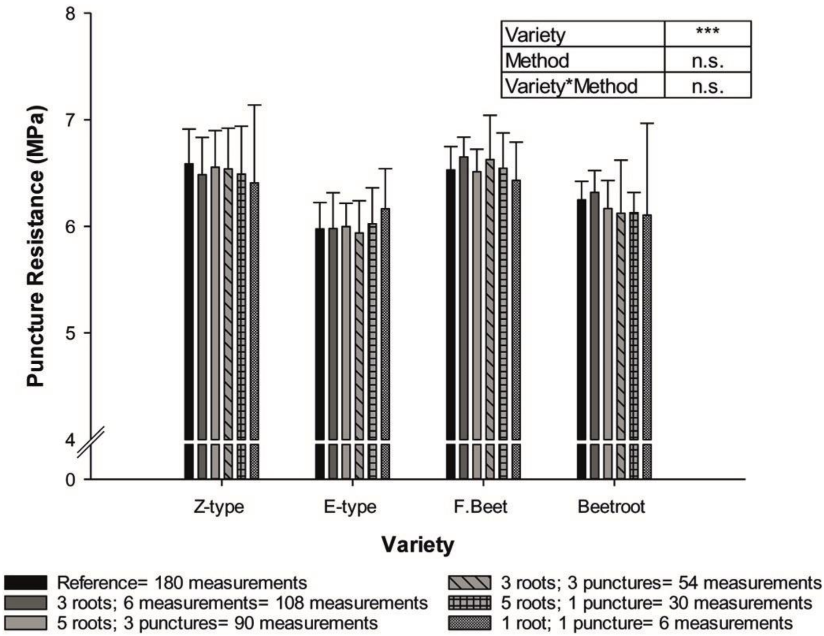


Fig. 8: Impact of the reduction of measurement repetitions on puncture resistance of four Beta varieties; 6 field replicates. Vertical bars indicate standard deviation. * indicate significant differences at p < 0.001, n.s. = not significant.**

From the reduction of the number of roots it could be demonstrated that the puncture and the compression tests provided stable results from measurement of only a few samples. The variability was slightly lower for the puncture test than for the compression test. This may be due to the described differences between the sample positions in the root. The number of roots included affected the results only marginally, which allows to get stable results with a relatively small sample size. Because of the singled plant population and the good growing conditions in 2017 the plots in the field trial had an even plant stand. In years with more unfavorable growing conditions and thus higher differences between single plots or between plants within a plot, it might be necessary to increase the sample size (number of roots). Also Nedomová et al. (2017) used only five sugar beet roots per treatment for the compression test, however, the higher coefficient of variation may point to a more uneven stand of the sugar beets.

In case of the puncture test it was tested whether it makes any difference to reduce the number of roots per plot or to reduce the repetition measurements per root. Results underline that the number of measurements had no significant effect, so that the mean value of 180 measurements was the same as for 1 measurement per plot.

The number of measurements per root influences mainly the accuracy of the measured value, while the number of single roots should represent the heterogeneity within the variety (or any other treatment). Since the goal of the puncture and compression test was to define the strength of a variety, the sample size should be adjusted to the heterogeneity of the plants, which means that in most cases it will be better to include a higher number of roots, even with less repetition measurements per root.

3.5. Field trial

3.5.1. Puncture test

The mean puncture resistance to penetrate the periderm differed between 5.98 MPa ± 0.25 MPa (E-type) and 6.86 MPa ± 0.57 MPa (beetroot) (Fig. 9 A). The value was influenced by variety and site. Furthermore, interactions between variety and site occurred. The E-type sugar beet variety had the lowest puncture resistance at both sites, while the highest was reached by beetroot at site 1. However, the standard deviation of the beetroot at that site was higher than for all other varieties, and at site 2 the beetroot had a significant lower resistance.

The tissue firmness was lower than the puncture resistance and differed between the varieties from 3.62 MPa ± 0.29 MPa (beetroot) to 5.34 MPa ± 0.30 MPa (Z-Type) (Fig. 9 B). Variety was the only factor, which had a significant influence on the tissue firmness, while there was no difference between the sites. The tissue firmness decreased from Z-type over E-type and fodder beet to beetroot.

With the puncture test it was possible to measure two different resistances within the root. The puncture resistance describes the periderm strength, while the tissue firmness indicates the strength of the outer tissue of the root. With both methods significant differences between the tested varieties were found. The tissue firmness gave a more stable value over the growing sites than the puncture resistance, so that differences among varieties can be better described more by tissue firmness.

3.5.2. Compression test

The compressive strength varied from 1.47 MPa ± 0.06 MPa (beetroot) to 2.65 MPa ± 0.10 MPa (Z-Type) (Fig. 9 C). The compressive strength was affected by variety and site, but a variety by site interaction was not found. The sugar beet Z-type had the highest compressive strength, the beetroot the lowest. The decrease in compressive strength was in the same order as the tissue firmness. However, the differences were significant for all varieties.

The compression test allowed to distinguish between all tested varieties. In contrast to tissue firmness, the compressive strength allowed to distinguish between the E-type and the fodder beet.

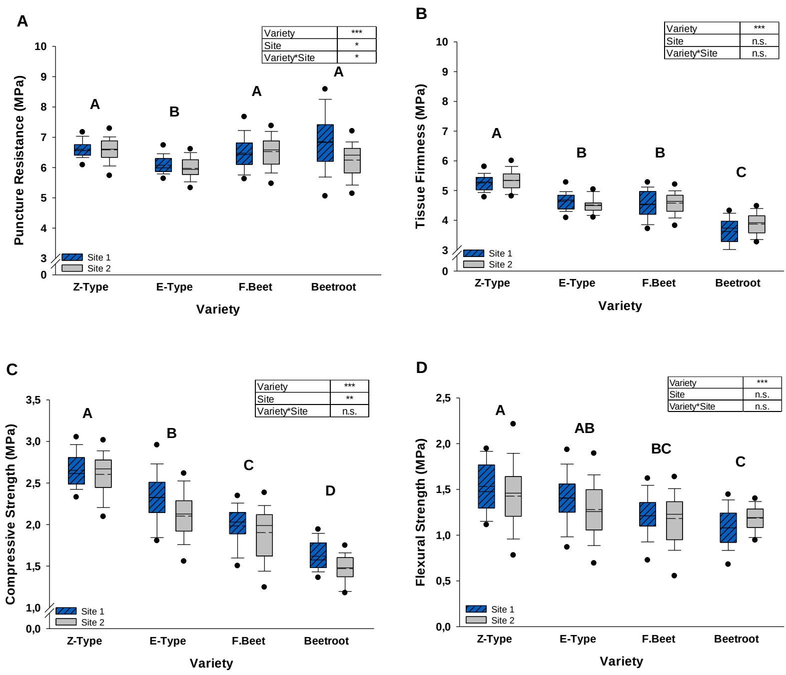


Fig. 9: Puncture resistance (A), tissue firmness (B), compressive strength (C) and flexural strength (D) of four Beta varieties at two sites.

Boxplots include 6 field replicates and 30 beets with 6 measurements for the puncture test, 3 sample positions for the compression test, and 2 samples for the flexural test, respectively. Different letters indicate significant differences in means between varieties. * indicate significant differences at $p < 0.001$, ** at $p < 0.01$, n.s. = not significant.**

Therefore, the compression test is considered to be a stable method for the description of sugar beet texture.

3.5.3. Flexural test

With the flexural test significant differences could only be found between some of the four tested varieties (Fig. 9 D). The flexural strength of beetroot was significantly lower than the one of the two sugar beet varieties. Furthermore, the fodder beet was significantly weaker than the Z-type. The order of the varieties regarding their flexural strength was similar to the order of the compressive strength and tissue firmness. The flexural test could only distinguish between the extreme varieties in this study (Z-type and fodder beet / beetroot). The high variability of the flexural strength within one plot could not be reduced while processing roots from the field trial. The sample preparation was rather time-consuming and complex, and it seems that even minimal differences in the size of the sample or injuries to the sample body can affect the measurement. Because of these difficulties the flexural test (bending) seems not to be suitable to precisely describe differences in the texture of different sugar beet varieties. In contrast to Drath (1976) the support span was reduced to decrease the area under pressure and thus the variability of the measurements. Also Drath et al. (1984) could not detect differences between varieties regarding their flexural strength.

4. Conclusion

The comprehensive tests could provide valuable information about sample preparation, measuring position, time of measurement, method and sample size for the texture analysis of Beta varieties. It has been demonstrated that hand washing of the roots before measurement did not influence the results. The roots should be measured preferentially within 48 h after harvesting and washing. However, the differences between the varieties were significant at any time of measurement. The sample position has an impact on the results of puncture and compression test. Therefore, the sample position has to be well defined before any measurement. For puncture testing it is recommended to distribute the measurement positions radially around the widest part of the root, omitting root groove and crown tissue. The compressive strength decreases from the outer to the inner part of the root. This characteristic course allows a comparison of varieties, treatments or studies only, when the sample position is clearly defined. Puncture and compression tests allowed a differentiation of the tested varieties regarding their tissue strength. A reduction of the sample size to 6 roots per variety was possible. However, if the plant stand of a field trial is unequal, a larger sample size is advised. The flexural test showed a very high variability among repetitions and is, therefore, not recommend for a characterization of sugar beet varieties.

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Manuskript II:

Tissue strength of sugar beet roots – genotypic variation and environmental impact[2]

Gunnar Kleuker and Christa M. Hoffmann

Abstract

Tissue strength of sugar beet (*Beta vulgaris L.*) roots could be an interesting breeding target, as it is a possible indicator for storability and influences the manufacturing process. The objective of this study was to analyse the importance of genotype and environment and their interaction on three texture parameters (puncture resistance, tissue firmness, and compressive strength) and to investigate the range of tissue strengths of commercial sugar beet genotypes. For that purpose, two trial series were conducted with 6 sugar beet genotypes in 7 environments across Germany in 2018 and 2019. A screening was performed with 12 commercial genotypes at one site in 2020. Tissue firmness and compressive strength were closely correlated with the puncture resistance of sugar beet roots. The genotype effect was distinctly higher for the texture parameters than for yield and quality parameters, while the genotype by environment interaction was negligible, allowing a selection based on few sites. For both genotype and environment, compression strength was closely related to the relative sugar content of the beets. Commercial genotypes also covered a wide range of tissue strengths. These differences might affect harvest damage, storage losses, and subsequent processing steps in the factory.

Keywords: Breeding, Compression, Genotype effect, Genotype-Environment-Interaction, Puncture

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1. Introduction

Tissue strength of fruits and vegetables is a critical parameter for consumer acceptance. It is measured in fresh and cooked products but is of special interest during storage of fresh fruits and vegetables, as their tissue strength changes during storage (Abbott 2004). The tissue strength of sugar beet is currently under discussion as it might influence harvest and processing quality. Tissue strength is the force required to destroy the sugar beet root and may thus represent its response to external impacts. In long-term storage trials the tissue strength of the root turned out as a possible indicator for the susceptibility of sugar beet genotypes to damage during harvest and for their storability (Kleuker and Hoffmann 2020; Nause et al. 2020). Harvest, especially lifting and cleaning of the root, is mechanical intensive and leads to injuries, which may act as entry points for pathogens during storage.

Tissue strength of sugar beet roots thus appears to be an interesting parameter regarding losses during sugar beet harvest and storage. However, that has never been investigated before. Last studies on tissue strength of sugar beet were already carried out in the 1970s and 80s, but with main focus on the impact on the slicing work in the factory (Drath 1976; Drath et al. 1984; Vukov 1977; Vukov 1975). Recently, in years with drought stress, sugar factories faced difficulties during cutting, extraction and especially pulp pressing (Frenzel 2021), indicating an altered tissue strength of the sugar beet roots. Therefore, tissue strength of sugar beet is possibly important for the beet growers, but definitely for the processors.

For sugar beet, Kleuker and Hoffmann (2019b) published the first comprehensive protocol to measure tissue strength in sugar beet through puncture and compression tests with a texture analyser. The puncture test measured the maximum resistance of the root periderm as puncture resistance and the strength of the underlying tissue as tissue firmness. They described a relation between the puncture resistance and the tissue firmness, both measured with a puncture probe, which was confirmed by Nause et al. (2020). As these studies were conducted at only two sites, the relationship between the parameters has to be tested across a wider set of environments. Furthermore, differences in compressive strength between the measuring positions within one beet were reported (Gemtos 1999; Senge and Hajinezhad 2009; Kleuker and Hoffmann 2019b). However, these authors came to different results and conclusions. A comprehensive review regarding the consistency of the differences between the measuring positions seems useful.

If tissue strength turns out to be important for variety performance of sugar beet, it will possibly be included into the official variety testing as a new trait. New sugar beet varieties are approved if the official testing attests a higher average yield, quality, or resistance in different environments than the existing varieties. However, this average performance of the varieties is only of importance if the varieties react similar or even equal across different environments. However, if a genotype by environment interaction (GEI) occurs, the genotypes respond differently in different environments (Annicchiarico 2002). The most pronounced case is the crossover reaction, which leads to a shift in the ranking of genotypes in specific environments (Baker 1988). Therefore, an interaction has to be taken into account for breeding and selection.

For yield and quality parameters of sugar beet, Hoffmann et al. (2009) investigated the genotype by environment interaction in 52 environments across Europe. They observed the GEI to be smaller than for other crops such as cereals (Ceccarelli 1989; 1994) and did not find any crossover interactions for yield and quality parameters pointing to a wide adaption to environments. Although Kleuker and Hoffmann (2020) found first evidence that also the tissue strength of sugar beet roots is environmentally stable, this has to be underlined in more comprehensive investigations quantifying the effect of genotype and environment.

The objectives of this study were (1) to validate the method of texture analysis in a wider range of environments, (2) to quantify the effect of genotype, environment and their interaction on tissue strength and (3) to evaluate the range of tissue strengths in commercial varieties.

2. Material and Methods

2.1. Field trials

Six sugar beet genotypes were grown at three sites in Germany (Eibelstadt (SZ), Göttingen (IfZ) and Hankensbüttel (NZ)) in 2018 and 2019. The trials were designed as completely randomized block designs with six replications (Trial A). In 2019 the trial at Hankensbüttel (NZ) was supplemented by an irrigation treatment, resulting in seven growing environments. Genotypes were selected based on their yield type according their characterisation of the German Federal Plant Variety Office (high sugar content, high root yield, balanced type) and their behaviour in previous storage trials (Hoffmann and Schnepel 2016a). The genotypes differed also in their year of registration from the German Federal Plant Variety Office, an old genotype was approved before 2000, while the others were approved between 2006 and 2017.

Crops were sown between end of March and mid of April according to soil and weather conditions. The environments differed in soil type and precipitation (Tab. 1). Fertilizer application and plant protection were carried out according to regional guidelines of good agricultural practice. Roots were machine harvested in October and were sent to Göttingen (IfZ) for quality and texture analysis.

Based on the results of the trials from 2018 and 2019, a field trial with 12 genotypes was conducted at the site IfZ in 2020 (Trial B) in a randomized block design with six replications. To investigate the range of tissue strength of sugar beet in Germany, genotypes 1-3 from Trial A were included as standard genotypes, as they represented all yield types and nine recent commercial genotypes (registration 2012-2019) were chosen considering the different genepools from five breeding companies (Tab. 2). Genotypes with different yield types according to the German Federal Plant Variety Office and breeders' classification were chosen. Roots were machine harvested in October and analyzed in Göttingen.

Tab. 1: Environmental characteristics of the field trials and yield and quality parameters of sugar beet (6 genotypes), Germany (2018 and 2019); different letters indicate significant differences within a column, Tukey-Test α= 0.05.

Year	Site	Env.	Soil-type	Seeding date	Harvest date	Precipitation Apr-Oct.	Irrigation	Root yield	Sugar content	Sugar yield	Puncture resistance	Tissue firmness	Compressive strength
						mm		t ha^{-1}	% Pol.	t ha^{-1}	MPa	MPa	MPa
2018	IfZ	IfZ18	Loam	19/04	15/10	245	-	73.2b	20.7a	15.1b	6.79a	5.39a	2.64a
2018	NZ	NZ18	Loamy Sand	07/04	05/10	243	90	48.8c	16.7d	8.2c	6.17b	4.66c	1.78d
2018	SZ	SZ18	Loam	07/04	04/10	276	-	72.7b	20.6a	14.9b	6.41ab	4.86b	2.35b
2019	IfZ	IfZ19	Loam	08/04	14/10	334	-	99.6a	19.1b	18.9a	6.52ab	5.31a	2.26bc
2019	NZ	NZ19	Loamy Sand	08/04	01/10	404	-	17.7d	16.2d	2.9d	5.72c	4.29c	1.53e
2019	NZ	NB19	Loamy Sand	08/04	01/10	404	120	54.1c	18.5bc	10.0c	6.48ab	4.83b	2.03c
2019	SZ	SZ19	Loam	28/03	01/10	293	-	91.2a	18.4c	16.8ab	6.41ab	5.12a	2.13c

Tab. 2: Characteristics of the commercial sugar beet genotypes and standard genotypes of the field trial in 2020 in accordance to the German Federal Plant Variety Office and breeders' classification.

Genotype	Yield type
1	root yield type
2	high sugar content
3	balanced type
4	high sugar content
5	balanced type
6	root yield type
7	balanced type
8	balanced type
9	balanced type
10	high sugar content
11	high sugar content
12	high sugar content

2.2. Quality analysis

After harvest the sugar beet roots were washed and processed in the tare house at the IfZ in Göttingen. For the determination of root yield, washed roots were weighed and afterwards processed to homogenous beet brei. The brei was shock frozen and stored at -20 °C until analysis.
The analysis of the brei filtered with 0.3% Al-sulphate solution was carried out with an automated beet laboratory system (Anton Paar OptoTex GmbH, Seelze). The sugar content was determined with a polarimeter following the routine methods of ICUMSA (1994).

2.3. Texture analysis

The tissue strength of the root was determined with a texture analyser (TA.XTplus 100, Stable Micro Systems, Godalming, UK). A 100 kg load cell was used to perform puncture and compression tests, with five roots per plot. Both tests were conducted with a crosshead speed of 60 mm min^{-1}. The method was extensively studied and adapted to sugar beet roots by Kleuker and Hoffmann (2019b). The term tissue strength was chosen to summarize the overall results of puncture and compression test.
After harvest, sugar beet roots were stored in a climate chamber at 6 °C for a maximum duration of seven days. One day prior to measurement, roots were washed and stored at room temperature (20 °C) overnight. The number of washed roots was adapted to the throughput of the texture analysis, so the roots were analysed within 24 hours after washing.
The puncture test was performed with a cylindrical steel probe (Ø = 2 mm). Three measurement points per root were arranged radially around the largest root diameter. Root crown and groove were omitted.

The puncture resistance of the periderm, which corresponds to the force required to break the periderm, and the tissue firmness, defined as the average resistance of the underlying tissue (1 - 5 mm), were determined.

Additionally, compression measurements were performed on cylindrical samples of the same root and the compressive strength, as force to destroy the cylinder, was determined. For this purpose, a slice of at least 2 cm height was cut from the widest root diameter using a bread slicer (Raadvad). Two cylinders with a diameter of 18 mm were cut with a cork borer and adapted to a height of 20 mm. The cylinders were taken from different regions of the slice: Cylinder 1 included half of the central cylinder of the root, cylinder 2 was taken from the outermost part of the root. Results show the difference between cylinders 2 and 1.

2.4. Statistics

The statistical analysis of the three texture parameters was performed with the program SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) on the plot mean values. Figures and regression analysis were made with SigmaPlot 14.0 (Systat Software Inc., San Jose, CA, USA). Data were checked for normal distribution and homogeneity of variance according to the recommendations by Kozak and Piepho (2018). The differences between measuring positions of the compressive test are presented in bar charts with standard deviation. Differences between the environments in yield, quality, and texture parameters were analysed with an ANOVA using SAS Proc Mixed with posthoc Tukey-Test with $\alpha \leq 0.05$.

To investigate the effect of the GEI on the three texture parameters, the variance components and the two determinants of the GEI were estimated according to Annicchiarico (2002). The GEI is determined by heterogeneity of genotypic variance due to a scale effect (Annicchiarico 2002) and by imperfect correlations or changes in rank of the genotypes among different environments (Muir et al. 1992). Consequently, the lack of genetic correlation is the important determinant of the GEI for the breeder. These determinants can be estimated through the formulae provided by Dickerson (1962) and reported by Cooper et al. (1996).

Through separate ANOVAs for the individual environments (j), the genotypic variance ($s_{g(j)}^2$) was estimated from the MS (mean square) of genotype ($M_{g(j)}$) and the experimental error ($M_{e(j)}$) as:

$$s_{g(j)}^2 = (M_{g(j)} - M_{e(j)})/r$$

r was the number of replicates in the environment. The ANOVAs were calculated using the SAS Proc Mixed function with restricted maximum likelihood (REML) method. The heterogeneity of genotypic variance was estimated by the variance of the square root values $V(s_{g(j)})$. Its proportion in GEI variation is:

$$(V(s_{g(j)})/s_{ge}^2) \times 100$$

The extent of the lack of genetic correlation between the environments was estimated as the difference between s_{ge}^2 and $V(s_{g(j)})$. The contribution of this component to the variation in GE interaction is

$$[(s_{ge}^2 - V(s_{g(j)}))/s_{ge}^2)] \times 100$$

The pooled genetic correlation (r_g) among environments was estimated as:

$$r_g = s_g^2/(s_g^2 + s_{ge}^2 - V(s_{g(j)}))$$

In contrast to heritability, pooled genetic correlation only considers the part of the genotype environment interaction that is relevant for the breeder (lack of genetic correlation). Values close to 1 reveal substantially uniform response of genotypes across environments.

The genotypic and environmental variance component estimation was conducted with SAS Proc Varcomp function using REML method. The lack of correlation among environments and the GEI estimate were set in relation to the genotype main effect s_g^2.

To further investigate the influence of environmental quality and genotype, represented by root yield and sugar content, on the three texture parameters, the mean values for the individual environments and genotypes were calculated and plotted against the total mean value.

3. Results

3.1. Texture Analysis

Puncture resistance and tissue strength of the roots were closely correlated with a coefficient of determination of 0.83 (Fig. 10). The coefficient of determination between puncture resistance and compressive strength was lower (0.56), but also significant. The coefficient of determination increases to 0.63 when genotype 2 is excluded (data not shown).

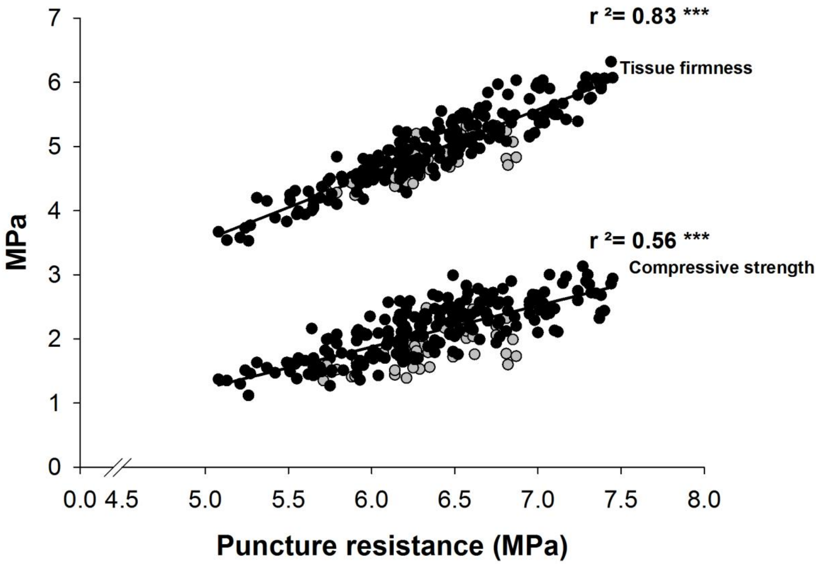

Fig. 10: Tissue firmness and compressive strength of sugar beet roots in relation to puncture resistance. 6 genotypes grown in 7 environments (site x year), Germany 2018 and 2019, n = 6 (Genotype 2 in grey) F-Test; ***= p≤ 0.001.

The mean difference between the two measurement cylinders of the compression test, taken from the outside and the inside of the root, was similar for all six genotypes (Fig. 11a). However, the difference between the measuring positions was significantly dependent on the environment. Differences were small in environments NZ18 and NZ19 and large in the other environments.

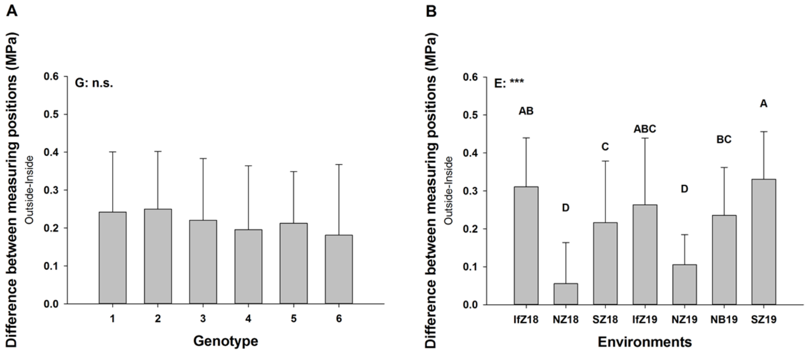

Fig. 11: Difference between measuring positions (Outside – Inside) in compressive strength of sugar beet roots; A: Genotypes (G) as mean of 7 environments (site x year) in Germany (2018 and 2019); B: Environments (E) as mean of 6 genotypes, n=6; Tukey-Test α= 0.05; ***= p≤ 0.001, n.s.= not significant, different letters indicate significant differences.

3.2. Multiple environment trial series

The seven environments differed not only in soil type, but also in precipitation (Tab. 1) resulting in significant differences in root yield, sugar content and sugar yield despite similar length of the growing season. Root yield in environment IfZ19 (99.6 t ha^{-1}) was more than five times higher than at NZ19 (17.7 t ha^{-1}). Low precipitation in environments NZ18 and NZ19 caused low root yield as well as low sugar content. In 2019, irrigation significantly increased root yield and sugar content, and consequently, sugar yield in the NB19 trial compared to NZ19.

Likewise, the three parameters of tissue strength differed significantly between the environments (Tab. 1). Sugar beet roots from IfZ18 had a puncture resistance of 6.79 MPa, whereas the roots from NZ19 had only a puncture resistance of 5.72 MPa. Tissue firmness (5.39 MPa to 4.29 MPa) and the compressive strength (2.64 MPA to 1.53 MPa) varied similarly. Irrigation at NB19 lead to an increased strength in all parameters in comparison to NZ19.

3.3. Genotype and environment effect

The variance component estimates for the texture parameters were mainly determined by environment and genotype. The influence of environment and genotype on puncture resistance and on tissue firmness was close (Tab. 3). In comparison, compressive strength was less influenced by the genotype (18.6%). The share of GEI was responsible for 2.3 to 3.4% of the total variance.

Lack of genetic correlation, the determinant of GEI relevant for breeding, accounted for 70.2 and 75.3% of the total GEI estimates of the puncture resistance and tissue firmness, respectively (Tab. 4). For the compressive strength the lack of genetic correlation accounted for only 45.5% of GEI.

The share of the total GEI on the genotype component ranged between 5.3 and 17.0% (Tab. 3), thus the lack of genetic correlation among environments, as part of GEI, was even smaller. Consequently, the pooled genetic correlation was high (≥93%).

In contrast, the variance component estimates of the yield parameters were predominately determined by the environment (>80%) (Tab. 3). The genotype effect was highest for the sugar content with 11.0% of the total variance, i.e. much smaller than for the texture parameters. The GEI was responsible for 2.5 to 4.1% of the total variance being 36.7 to 61.4% of the genotype effect. Relation of tissue strength and root yield and sugar content

Tab. 3: Variance components of texture and yield parameters of sugar beet; 6 genotypes; 7 environments (site x year) in Germany (2018 and 2019).

	% of total variance					
	Environment (E)	**Genotype (G)**	**GEI**	**Rep. (E)**	**Error**	**GEI / G (%)**
Puncture resistance (MPa)	38.0	36.2	3.4	9.8	12.5	9.5
Tissue firmness (MPa)	41.2	43.1	2.3	4.2	9.2	5.3
Compressive strength (MPa)	65.5	18.6	3.2	3.3	9.5	17.0
Root yield (t ha^{-1})	86.8	6.4	2.5	2.9	1.5	39.6
Sugar content (% Pol.)	80.1	11.0	4.1	1.9	2.9	36.7
Sugar yield (t ha^{-1})	89.2	2.5	2.5	3.0	1.2	61.4

Tab. 4: Determinant of genotype environment interaction for three tissue parameters according to Annicchiarico (2002); 6 sugar beet genotypes, 7 environments (site x year) in Germany (2018 and 2019).

	Proportion of the GE interaction variance accounted for		
	heterogeneity of genotypic variance (%)	**lack of genetic correlation among environments (%)**	**pooled genetic correlation r_g**
Puncture resistance	29.8	70.2	0.94
Tissue firmness	24.7	75.3	0.96
Compressive strength	54.5	45.5	0.93

Figure 12A shows the relative compressive strength of each genotype and environment in relation to the relative root yield. The relationship differed for environments compared to genotypes. In environments, where a high relative root yield was achieved, roots also featured a high relative compressive strength. In contrast, when comparing genotypes, the relative compressive strength tended to decrease with increasing relative root yield.

The relation of the compressive strength with the relative sugar content showed a similar effect for environments and genotypes (Fig. 12B). With increasing relative sugar content the compressive strength increased. An exception was the genotype 2 with a high relative sugar content, but low relative compressive strength. The results for the two puncture parameters were similar (data not shown).

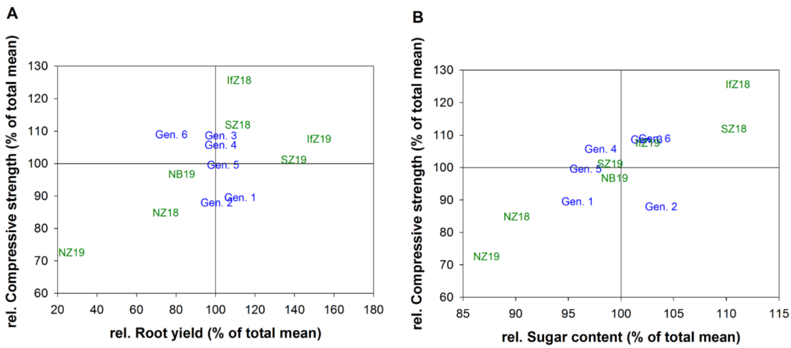

Fig. 12: Relative root yield (A) and sugar content (B) in relation to compressive strength for 6 sugar beet genotypes grown at 7 environments (site x year) in Germany 2018-2019, n=6.

3.4. Tissue strength of commercial genotypes

The puncture resistance of the nine commercial genotypes in relation to the three standard genotypes (1, 2, and 3) from the multi environment trial is shown in Fig. 13A. Most of the genotypes (4-9) had a similar puncture resistance as genotypes 2 and 3. Genotype 1 showed the lowest puncture resistance, similar to the results from the multi environment trial. Genotypes 10, 11, and 12 had a considerably higher puncture resistance than the other tested genotypes.

Genotypic differences were greater in tissue firmness than in puncture resistance. Genotypes 2-9 had a similar relative tissue firmness, with genotype 4 having a slightly higher firmness and genotype 6 having a lower firmness (Fig. 13B). Genotypes 10, 11, and especially 12 had a higher firmness compared to the other genotypes.

Genotype 2 had the lowest relative compressive strength, followed by genotype 1 (Fig. 13C). Due to the low compressive strength of two of the three standard genotypes, all other genotypes had a relative compressive strength above 100%. However, compressive strength of genotypes 4, 10, 11 and 12 was considerably higher than the compressive strength of the other genotypes. Thus three rough groups could be formed, below 100%, between 100 and 110%, and above 120% relative compressive strength.

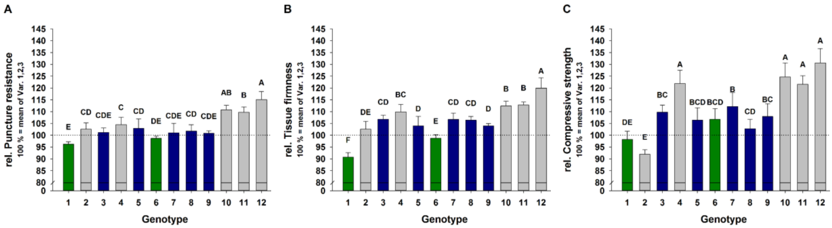

Fig. 13: Relative puncture resistance (A), tissue firmness (B) and compressive strength (C) of sugar beet roots, 100 = mean of standard genotypes 1, 2, and 3; 1 environment in 2020, n = 6; colours represent yield type according to the German Federal Plant Variety Office and breeders' classification (Green: root yield type, Grey: high sugar content, Blue: balanced type) TukeyTest α= 0.05; different letters indicate significant differences.

4. Discussion

While there is an assessment of yield and quality of sugar beet in the official variety testing, any parameter regarding root tissue strength is not considered so far. However, the strength of root tissue from freshly harvested roots could gain importance as a simple indication for damage susceptibility and the storability of sugar beet (Nause et al. 2020; Kleuker and Hoffmann 2020). Therefore, apart from analyzing the effect of genotype and environment, a first assessment of the range of root tissue strengths in different environments in Germany is presented in this study.

4.1. Texture Analysis

Even though the measurement of tissue strength with a texture analyser is a standard method for fruits and foods (Abbott 1999, 2004), it is relatively new for sugar beet. Thus, there is no information on possible interactions between different measuring methods and/or measuring positions and environments. Kleuker and Hoffmann (2019b) and Nause et al. (2020) described a close correlation between puncture resistance and tissue firmness in sugar beet roots and suggested to reduce the measurements to one parameter. Puncture resistance was regarded as the most meaningful parameter as it describes the rupture of the periderm, which offers an entry for pathogens during storage. In the current study, the close correlation between puncture resistance and tissue firmness was confirmed over a broad range of environments. Since both are measured in the same run and just describe the strength of the outer root at different depths, a close correlation was expected. It emphasizes the view that it is possible to reduce the measurements to puncture resistance only without loss of information.

Compressive strength was not as closely related to puncture resistance. Kleuker and Hoffmann (2020) discussed possible reasons for the differences between puncture and compression measurements, such as the direction of the measurement and the area of the analysed cell complex. However, it was also due to the differing behaviour of genotype 2 in compressive strength, which was characterized through a hard periderm, but a weak compressive strength. This tendency was confirmed when comparing 12 genotypes. The difference between those measurements suggests to determine both parameters to achieve a more comprehensive description of the tissue strength of the whole root.

The decline in compressive strength from the outside to the centre of the sugar beet root confirms results of Kleuker and Hoffmann (2019b) and Senge and Hajinezhad (2009). Interestingly, the decline from the outer to more inner tissue was similar for the different genotypes. A possible reason might be the characteristic structure of the sugar beet root which is defined by several cambium rings with xylem and phloem (Artschwager 1926a). During growth, the distance between the inner rings expands in particular and thus the fraction of parenchyma tissue in comparison to the outer parts of the root with a higher portion of vascular tissue, causing a lower compressive strength. The compressive strength must thus always be measured at the same position when comparing genotypes, but it is not necessary to measure at both inner and outer position.

On the contrary, the environment had a clear effect on the difference between the measuring positions. Opposite to all other environments, the environments NZ18 and NZ19 did not show big differences between the measuring positions. The reason might be low overall compressive strength as a consequence of drought stress, which particularly restricts the expansion of the inner cambium rings (Hoffmann 2010). Thus, the cell size, which is normally larger in the inner part of the root is also reduced and more similar to the outer cell size (Nause et al. 2020).

It seems that differences in compressive strength between the measuring positions only develop during undisturbed storage root growth. The need to measure both positions, therefore, depends on the objective. If genotypes are to be distinguished, it is sufficient to focus on one position. But if roots from different environments are studied more closely, the measurement of both positions seems to provide extra information.

4.2. Multiple environment trial series

The tissue strength of sugar beet roots was studied in several environments clearly differing in growing conditions, indicated by the distinct differences in root yield and sugar content. In sugar beet, moderate drought stress causes an increase of the sugar content because of the loss of water (Bloch et al. 2006); as at the sites IfZ18 and SZ18. However, severe drought stress reduces sugar accumulation and thus sugar content because of sincere metabolic disturbance (Hoffmann 2010a); as found in the environments NZ18 and NZ19 with low root yield and low sugar content. Obviously, the tissue strength decreased under severe drought stress, too.

The main reason for low root yield under severe drought stress is a decrease in cell expansion, as Hoffmann (2010a) confirmed for sugar beet in a comparison of drought stressed and well-watered roots. Drought also affected the relation of root yield and tissue strength: Low average yield was associated with low tissue strength in the extreme environments NZ 18 and 19, while in the other environments with largely normal growing conditions tissue strength was not as clearly related with root yield. By contrast, the genotype effect was opposite: With increasing root yield, the tissue strength tended to decrease. Therefore, root yield seems not to be the decisive parameter for tissue strength.

On the other hand, the close relation between sugar content and tissue strength points to a causal relation for both genotypes and environments. This might be explained with findings of Milford (1973a) who claimed that genotypes that differ in yield formation differ in cell size. In his explanation genotypes with higher sugar content have smaller cells, resulting in a higher cell wall to cell volume ratio. This assumption was confirmed by Hoffmann et al. (2005) and Hoffmann et al. (2018b) who found a high marc content (water insoluble cell wall compounds) in varieties with high sugar content. Furthermore, the results of the commercial genotype trial also emphasise the hypothesis that Z-type genotypes (10, 11, and 12) are stronger in their tissue. Accordingly, for potatoes and carrots Hudson (1975), Konstankiewicz and Zdunek (2001) and Zdunek and Umeda (2005) reported that greater force was necessary to bruise varieties with smaller cells.

This hypothesis that higher tissue strength is associated with higher cell wall content needs to be verified in further studies. Furthermore, it has to be investigated if a higher cell wall content is also accompanied by a different cell wall composition. Recently, Nause et al. (2020) analysed the tissue composition of sugar beet genotypes. They came to the conclusion that the total amount of cell wall compounds is more important for the tissue strength than the composition. But in their study only one environment was included, so that they did not focus on possible environmental impacts.

Our extensive study gives an estimation of the puncture resistance, tissue firmness and compressive strength of sugar beet roots over a range of extremely different environments. The data can serve as a benchmark for tissue strength of sugar beet roots in future experiments.

4.3. Genotype and environment effect

The genotypic effects on texture parameters, in particular puncture resistance and tissue firmness, were extraordinarily high with 36 and 43%. , while the environmental share was small in comparison to yield parameters. This is in line with Hoffmann et al. (2009) who reported a similar environmental effect on sugar beet yield and quality of more than 80%. The greatest genotype effect they found in eleven parameters under study was 8.5% for betaine. Therefore, texture parameters are the traits with the highest genotype effect and thus highest heritability determined for sugar beet so far.

The main explanation for the high genotype effect in sugar beet is probably, that the formation of the root structure through the cambium rings takes place during early development (Artschwager 1926a; Milford 1973a). Furthermore, sugar beet stays in the vegetative growth stage until harvest. Hence, root structure is formed very early and is therefore more determined by the genotype than by environmental changes during the growing season.

The subdivision of the GEI in lack of genetic correlation and heterogeneity of variance allows a more precise evaluation of the breeding relevant part of GEI. Atlin et al. (2000) and Annicchiarico (2002) suggested for genotype evaluation not to subdivide regions if the ratio of GEI to genotypic variance is below 0.3 or if the lack of genetic correlation variance is below 25-30% of genotypic variance. Since the estimated values for texture parameters were below those thresholds, the GEI for sugar beet root strength is negligible.

According to Kang (1997) a genotype by environment interaction tends to be greater when genotypes and environments vary widely. But despite the considerable differences between the environments and the choice of different genotypes in the current study, the genotype by environment interaction for sugar beet root strength was low. Because of this low GEI in combination with the strong genotype effect, even a single trial site seems to be sufficient to get a first impression of the tissue strength of commercial sugar beet genotypes.

4.4. Tissue strength of commercial genotypes

The texture analysis of commercial genotypes in comparison to standard genotypes 1, 2, and 3 of the previous trial series provides a first insight into the range of tissue strength. The genotypic range increased from puncture resistance, via tissue firmness to compressive strength, hence from the outside to the inside of the beet. Surprisingly, the commercial genotypes differed distinctly in their tissue strength covering a similar range as the genotypes in the first trial series, which were specially chosen with regard to differences. Although tissue strength has not been considered in breeding and registration so far, this points to a great potential for selection.

However, it is not yet clear to what extent these differences of the genotypes in tissue strength may influence the harvest and processing of the roots. Nause et al. (2020) and Kleuker and Hoffmann (2020) found first indications that a higher genotypic tissue strength decreases damage susceptibility and thus storage losses. For the processing of sugar beet in the factory there is no current information available if genotypes with weaker or stronger tissue are advantageous during slicing and pulp pressing. Vukov (1977) stated that both extremes lead to problems in processing, but without providing data from different genotypes.

5. Conclusions

The tissue strength of sugar beet roots was mainly determined by the genotype, less by the environment, with no relevant interactions occurring. The considerable differences between the commercial genotypes lead to the assumption that the tissue strength will affect sugar beet harvest and following processing steps in the sugar factory. However, the effect of storage on the tissue strength of sugar beet roots has to be further investigated, as a proportion of the sugar beet crop will be processed after a storage period. In order to alter the tissue strength by breeding, future studies will have to investigate the effects and the determinants of differences in tissue strength. Then the target value of tissue strength has to be defined, but it can possibly differ for sugar beet cultivation and processing.

Conflict of interest

There is no conflict of interest.

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Manuskript III:

Causes of different tissue strength, changes during storage and effect on the storability of sugar beet genotypes[3]

Gunnar Kleuker, Christa M. Hoffmann

Abstract

Genetic variation in the tissue strength of sugar beet (*Beta vulgaris L.*) roots has been found in recent studies. There are indications that tissue strength influences damage susceptibility and storability. The objective of this study was to analyse the impact of storage on tissue strength, to determine causes for differences in tissue strength and to find possible relations between tissue strength, damage susceptibility and storability of sugar beet genotypes. For this purpose, trials with six sugar beet genotypes were conducted in seven environments across Germany in 2018 and 2019, followed by a screening trial with 12 commercial genotypes at one location in 2020. Tissue strength changed during storage depending on the growing environment, but independently of the genotype; puncture resistance increased by 0.35 MPa. The genotypic tissue strength was mainly determined by the cell wall content (r^2 up to 0.97), less by the cell wall composition. For sugar beet genotypes, the relationship between tissue strength and storability could be explained by the fact that root tip breakage and subsequent storage losses tended to decrease with higher tissue strength, as shown by principal component analysis (PCA). Introducing tissue strength as a variety trait in breeding and official variety trials could thus contribute to reduced harvest and storage losses in the future.

Keywords: Storage, Damage, Sugar beet, Mechanical property

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1. Introduction

Sugar beet (Beta vulgaris L.) roots are usually stored between harvest and processing. During storage, sugar beet roots lose sugar due to respiration, wound healing, and pathogen infestation and the processing quality declines. As the processing campaigns in European sugar factories should be extended, the storage period and the amount of roots to be stored will also increase. Longer storage periods increase the risk of higher sugar and quality losses (Kenter et al. 2006; Campbell and Klotz 2007). During storage, the sucrose is cleaved, mainly by sucrose synthase, and invert sugar (glucose + fructose) is accumulated as function of time and temperature (Klotz and Finger 2004). As invert sugar reduces the alkalinity of the juices and leads to colour formation, it has detrimental effects during processing (van der Poel et al. 1998).

An important factor affecting storability, besides storage time and temperature, is mechanical damage during harvest (Mumford and Wyse 1976; Klotz et al. 2006). Mechanical damage increases energy demand for wound healing, but is also a potential entry point for pathogens as the intact cell wall barrier is destroyed (Wyse 1978; Bugbee 1982). The extent of mechanical damage is mainly determined by the harvester setting (Hoffmann et al. 2018a). Nevertheless, genotypic differences in damage susceptibility were found in trials by van Swaaij et al. (2003) , Hoffmann and Schnepel (2016a), but also in commercial field clamps (Hoffmann 2018). With decreasing root tip breakage, pathogen infestation during storage is lower (Hoffmann und Schnepel 2016a). Wiltshire and Cobb (2000b) concluded that there is little understanding of the factors influencing the susceptibility of sugar beet roots to damage. But a number of factors, such as turgor, tissue strength, and cell wall, have been identified for other crops. Hoffmann und Schnepel (2016a) and Schnepel and Hoffmann (2016) demonstrated that sugar beet genotypes with a high marc content (water insoluble cell wall components) had lower root tip breakage and pathogen infestation than those with low marc content. This resulted in lower storage loss. They assumed that these differences in cell wall content and perhaps also cell wall stability will cause differences in the susceptibility to pathogens.

Although there were some indications that tissue strength differs among sugar beet genotypes, studies of tissue strength are rare. Previous studies with the focus on the processing quality of sugar beets were conducted with different tools to determine the elasticity and cutting resistance (Vukov 1977; Drath 1976; Drath et al. 1984). Vukov (1977) gave a detailed summary of the various factors affecting the physics and chemistry of sugar beets and the impact on processing.

More recent studies were conducted with a texture analyser by Gemtos (1999), Gorzelany and Puchalski (2000), and Nedomová et al. (2017). A reliable method description for texture analysis of sugar beet roots was published by Kleuker and Hoffmann (2019b). Recently Kleuker and Hoffmann (2021a) showed that the tissue strength of sugar beet genotypes is an environmentally stable trait.

Sugar beet roots change in composition during storage. It can be assumed that these changes affect also the tissue strength / mechanical properties. Gorzelany and Puchalski (2000) and Nedomová et al. (2017) investigated the effect of storage on sugar beet tissue strength. Both found significant changes in tissue strength, but with contrasting results. In addition, there is no information about the possible causes and the effects of differences in tissue strength due to storage. A cause for differences in tissue strength might be the cell wall content. Sugar beet genotypes with high cell wall content, showed a lower damage susceptibility and thus might have a higher tissue strength than genotypes with low cell wall content (Hoffmann and Schnepel 2016a). Although there is some evidence regarding the effect of tissue strength, there has not yet been a comprehensive study examining the relationship between tissue strength of sugar beet roots and damage susceptibility and storage losses. The objective of this study was (1) to investigate the effect of storage on tissue strength, (2) to find reasons for different tissue strengths between sugar beet genotypes, and (3) to test whether tissue strength could be an indicator of sugar beet root storability.

2. Material and Methods

2.1. Field trial

In 2018 and 2019 six sugar beet genotypes were grown at three sites in Germany. The sites were located in the main German growing regions, at Eibelstadt (SZ), Göttingen (IfZ) and Hankensbüttel (NZ)). The trial design was a randomized block design with six replications. In Hankensbüttel 2019, two identical trials were sown; one trial was with irrigation (NB19) and the other without irrigation (NZ19). A total of seven environments (site x year x irrigation treatment) were included in this study (S1). Due to the highly diverse soil and weather conditions, these environments are characteristic for the sugar beet growing regions in temperate climates. The genotypes differed in their yield type (Z-type: high sugar content, N-type: high root yield, NZ-type: balanced type) and their behaviour in previous storage trials (differences in invert sugar content after storage) (Hoffmann and Schnepel 2016a). Genotype 6 was an old variety with registration of the German Federal Plant Variety Office before the year 2000, while the other genotypes were approved between 2006 and 2017.

Sugar beet were sown between end of March and mid of April dependent on local soil and weather conditions. Plots were between 10 and 15 m2 with a plant population of 80.000 to 100.000 plants per ha. Plant protection and fertilizer management were carried out according to guidelines of good agricultural practice (Landwirtschaftskammer Niedersachsen 2020). Sugar beet roots were machine harvested in October and sent to Göttingen for texture and quality analysis and storage treatments.

In 2020 a field trial with 12 genotypes was conducted in a randomized block design with six replications near Göttingen (IfZ20). Genotypes 1-3 were the same as in 2018 and 2019 and chosen as standard genotypes, as they were still commercially available. The other genotypes were recent commercial genotypes (registered between 2012 and 2019) and chosen considering different gene pools and yield types.

2.2. Storage Trials

The roots from each plot were divided into a reference and a storage sample. Except for the samples from the NZ19 environment, the storage sample consisted of two air-permeable potato bags weighing approximately 20 kg each. Due to the low yield at NZ19, only one bag was stored. Therefore, it was not possible to perform a texture analysis after storage. The storage samples were stored in a climate container 7 9 °C and a relative humidity of 99 % for 11 to 13 weeks (S1). Roots from SZ18 were stored for only 8 weeks, as they already showed severe rot and mould symptoms. Storage samples were covered with an additional layer of roots to reduce the position effect through transpiration (Schnepel and Hoffmann 2016).

2.3. Texture analysis

After harvest and after storage five beets per plot were used to analyse the tissue strength with a texture analyser (TA.XTplus 100, Stable Micro Systems, Godalming, UK). Equipped with a 100 kg load cell, puncture and compression tests were performed. Both tests were conducted with a cross-head speed of 60 mm min^{-1}. The procedure followed the description by Kleuker and Hoffmann (2019b), who adapted the texture analysis to sugar beet roots. The term tissue strength should jointly describe the results of puncture and compression test.

Texture analysis was conducted as soon as possible, but in some cases it took up to 7 days from harvest to measurement. In the meantime, the roots were stored in a climate chamber at 6 °C. A day before the texture analysis, roots were gently washed and stored overnight at room temperature (20 °C). The number of washed roots was adapted to the throughput of the texture analysis, so that a measurement within 24 h after washing was guaranteed.

The puncture test was performed with a cylindrical probe (Ø= 2 mm) at three measurement points per root; arranged radially around the largest root diameter. Root crown and groove were omitted. The puncture resistance (Pmax), which corresponds to the force required to penetrate the periderm, and the tissue firmness (Pmean), defined as the average resistance of the underlying tissue until a depth of 5 mm, were determined. Additionally, the distance (in mm) to rupture of the periderm (Pdist) was recorded.

With the same roots additional compression tests were performed. The compressive strength (Cmax), as the force to destroy the cylinder, and the distance (in mm) to the point of rupture (Cdist) were determined. For sample preparation a slice of at least 2 cm was cut off at the widest root diameter using a guillotine bread slicer (Raadvad, Denmark). Two cylinders were cut out with a cork borer with a diameter of 18 mm and shortened to 20 mm height. The cylinders were taken from different areas of the slice: Cylinder 1 included half of the central cylinder of the root, while cylinder 2 was taken from the outermost part of the root.

2.4. Fiber analyses

For the determination of the cell wall content, the alcohol insoluble residues (AIR) were determined through successively removing soluble components of the beet brei by washing in 95 % ethanol for two times (McFeeters and Armstrong 1984). AIR was dried at 105 °C for 24 hours to constant mass. From AIR, cell wall composition was quantified by the solubility of cell wall fractions in different detergents using a manual FibreBag System (C. Gerhardt GmbH & Co. KG, Germany) and detergents in accordance to (VDLUFA 2012a, 2012b, 2012c). These included soluble and insoluble pectin, hemicellulose, cellulose, and lignin. The single components are expressed as percentage of AIR, while AIR is expressed as percentage of root fresh mass.

Marc, which includes all water insoluble cell wall compounds, was determined in beet brei through successively removing the soluble components with boiling water, which was repeated four times. In the last step the insoluble residues were washed with acetone, then dried at 105 °C to constant weight (Reinefeld and Schneider 1983a). The marc content is expressed as percentage of root fresh mass.

2.5. Quality analysis

From the washed roots a homogenous brei was prepared and subsequently shock frozen at -40 °C and stored at -20 °C. Before analysis, the brei was mixed with 0.3 % aluminium sulphate solution for clarification. The sugar content was determined polarimetrically with an automated beet laboratory system (Anton Paar OptoTex GmbH, Seelze) in accordance to ICUMSA (1994). Potassium, Sodium and α-amino-N (blue number method) were analysed according to ICUMSA method procedure (ICUMSA 2007a, 2007b). Glucose content was determined with immobilised enzymes (Firma Dr. Müller, Freital, Deutschland; ICUMSA 2019) and converted to invert sugar by the factor 1.735 according to Vermeulen (2015). Invert sugar accumulation was calculated as the difference in invert sugar content after storage and before storage and is expressed on fresh mass basis. Dry matter (DM) was determined by oven-drying at 105 °C for 24 h. The widest diameter of the root tip breakage was measured at ten machine harvested roots per plot with a ruler and was used as an indicator for damage susceptibility.

2.6. Statistics

All calculations were performed on plot mean values. Data were checked for normal distribution and homogeneity of variance according to Kozak and Piepho (2018), violations were transformed by a boxcox transformation using the SAS Proc Transreg function (SAS 9.4, SAS Institute, Inc., Cary, NC, USA). Subsequently an analysis of variance was conducted with the Proc Mixed function (REML) with posthoc Tukey-Test with $\alpha \leq .05$. Figures and regression analysis were made with

SigmaPlot 14.0 (Systat Software Inc., San Jose, CA, USA). The genotypic, environmental and storage variance component estimation for the trials of 2018 and 2019 was conducted with Proc Varcomp function using REML method. To determine latent variables and to reveal interrelationships within the data of 2018 and 2019, a principal component analysis was conducted with Proc Factor function. Apart from the cell wall composition data, the presented prestorage data were used to calculate PCA. Cell wall composition data was excluded, as the differences were only small. The first 2 PCs were retained due to scree test (Cattell 1966) and were subject to the orthogonal rotation (Varimax). To visualize the relations, the factor loadings are plotted as a bivariate graph, with the centre being the overall mean of the data 0, since the data were normalized to the mean. The correlation is given by the angle to the centre.

3. Results

3.1. Texture analysis

Puncture resistance, tissue firmness and compressive strength before and after storage are shown in Fig. 14a for each environment. The effect of the environment on all three parameters was similar: strong drought stress reduced tissue strength (NZ18 & NZ19), while irrigation at NB19 increased it compared to NZ19. The effect of storage was similar for puncture resistance and compressive strength as for both the required force was higher after storage than at harvest. In contrast the tissue firmness hardly changed or even decreased slightly. The tissue strength parameters of the genotypes showed similar tendencies for the storage effect as there was no interaction between genotype and storage (Fig. 14b, Tab 5). While genotype 6 had in all three parameters the strongest tissue, genotype 1 had always the weakest. Overall, the two test methods (puncture and compression) showed similar results, only genotype 2 had a low compressive strength in relation to its puncture test results.

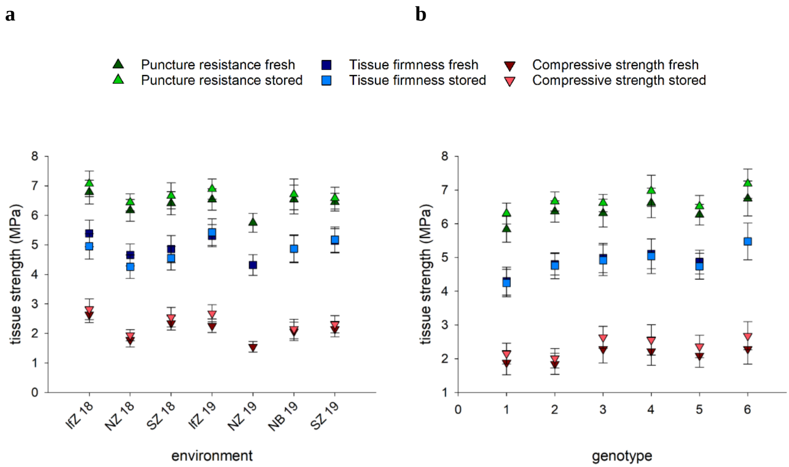

Fig. 14: Puncture resistance, tissue firmness and compressive strength of sugar beet roots before and after storage in climate containers; a: Environments as mean of 6 genotypes (NZ 19 and NB 19 same site; NB 19 with irrigation); b: Genotypes as mean of 7 environments (Germany 2018+19); n=6; symbols represent the mean value, whiskers the standard deviation.

The distance to periderm rupture (Pdist) of fresh roots was similar in all environments (Fig. 15a). Whereas the response to storage differed. While the distance to rupture increased considerably after storage in 2018, the change was much smaller in 2019. The distance to rupture in the compression testing (Cdist) differed strongly between the environments. The Cdist was noticeably lower in the drought stressed environments NZ18 and NZ19 than in the other environments. The genotypic difference in the distance to rupture of the periderm was also small and the reaction to storage was similar for all genotypes. There were small genotypic differences in the distance to rupture by compression testing where genotype 2 had a lower distance. The variance component analysis (Tab. 5) confirmed that storage had a strong influence on Pdist, but a lower impact on puncture resistance (Pmax) and compressive strength (Cmax). An interaction between environment and storage was found only for tissue firmness and distance to periderm rupture. However, no interaction between genotype and storage was found, i.e. all genotypes responded similarly to storage.

Tab. 5: Variance components of texture parameters of sugar beet; 6 genotypes (gen.); 7 environments (env.) in Germany (2018 +19); 2 storage treatments (fresh and stored); Pmax=Puncture resistance, Pmean= Tissue firmness, Pdist= Distance to rupture of periderm, Cmax= Compressive strength, Cdist= Distance to rupture by compression testing.

Variance Components	Pmax	Pmean	Pdist	Cmax	Cdist
	% of total variance				
environment	33.5	35.6	6.6	55.8	74.4
genotype	30.1	40.1	2.2	20.2	9.4
gen. x env.	4.9	2.0	0.3	1.9	1.7
storage	9.7	1.4	52.6	7.9	0.7
env. x stor.	0.7	8.4	21.3	1.8	1.5
gen. x stor.	0.6	0.0	0.3	0.6	0.3
gen. x env. x stor.	0.0	0.0	3.6	0.6	0.5
block(env.)	7.5	3.1	0.3	2.8	2.8
error	13.1	9.4	12.9	8.5	8.7

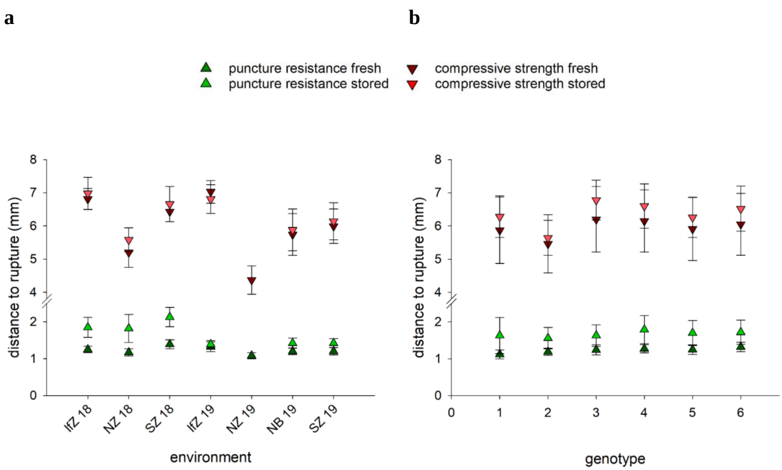

Fig. 15: Distance to rupture of the puncture test and compressive test of sugar beet roots before and after storage in climate containers; a: Environments as mean of 6 genotypes (NZ 19 and NB 19 same site; NB 19 with irrigation); b: Genotypes as mean of 7 environments (Germany 2018+19); n=6; symbols represent the mean value, whiskers the standard deviation.

3.2. Dry matter fractions

The dry matter content of the root varied greatly between the environments. In environments with drought stress (NZ18 and NZ19) roots had significantly lower dry matter content (Fig. 16a). In 2018, the dry matter content increased during storage, but in 2019 it hardly changed, except for NZ19 where it decreased. Sugar beet root dry matter is mainly determined by sugar content, obvious from the consistent behaviour of the two parameters. The sugar content was significantly lower at NZ18 and NZ19 compared to the other environments.

However, storage had less effect on sugar content than on dry matter. The sugar content did not change considerably in environments IfZ18 and IfZ19, while it decreased in the other environments during storage. The AIR content was reduced in environment NZ18 compared to the other 2018 environments, whereas an increased cell wall content (AIR and marc) was observed for sugar beet roots from environment NZ19 in comparison to NB19. Thus, Irrigation in NB19 reduced AIR, while the sugar content increased. This effect contrasted with the other environments, where a high sugar content was associated with high cell wall content. The AIR and marc content behaved similarly in the different environments.

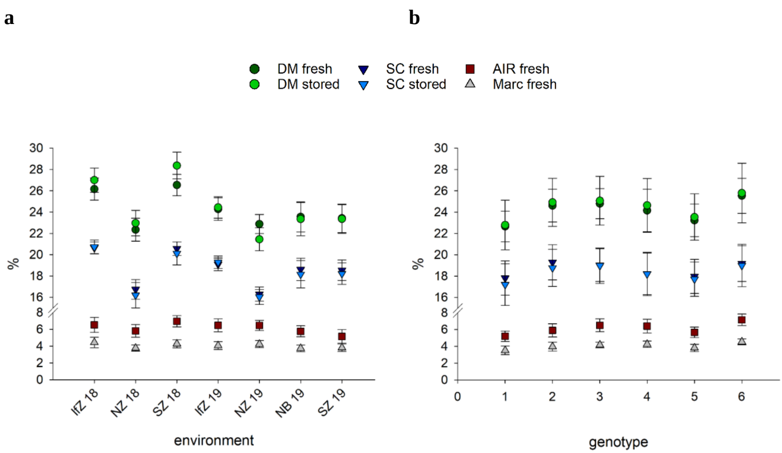

Fig. 16: Dry matter (DM)and sugar content (SC) of sugar beet roots before and after storage in a climate container, alcohol insoluble residues (AIR) and water insoluble residues (marc) of sugar beet roots after harvest (fresh); all data on fresh mass basis; a: Environments as mean of 6 genotypes (NZ 19 and NB 19 same site; NB 19 with irrigation); b: Genotypes as mean of 7 environments (Germany 2018+19); n=6; symbols represent the mean value, whiskers the standard deviation.

Significant differences in the dry matter content were also found for the genotypes (Fig. 16b). Genotype 6 had the highest dry matter content, genotype 1 the lowest. Similar results were found for the sugar content, with genotype 2 having the highest sugar content, followed by genotype 6. While genotypes 3 and 4 showed almost no change in sugar content during storage, for the other genotypes, especially genotypes 1 and 2, the sugar content was reduced. The highest AIR and marc contents were obtained for genotype 6. In general, AIR content was high for varieties with high sugar content, only variety 2 had a relatively lower AIR content.

Analysis of the different proportions of the cell wall components pectin, hemicellulose, cellulose, and lignin in AIR based on their solubility in various agents showed that the environmental effect was small, but significant for all cell wall components (Fig. 17a). On average, AIR was composed of 60 % pectin, 20 % hemicellulose, 14 % cellulose, and 6 % lignin. Differences between genotypes were observed for the pectin and hemicellulose content, with genotypes 3, 4, and 6 having higher pectin content than genotype 1 (Fig. 17b).

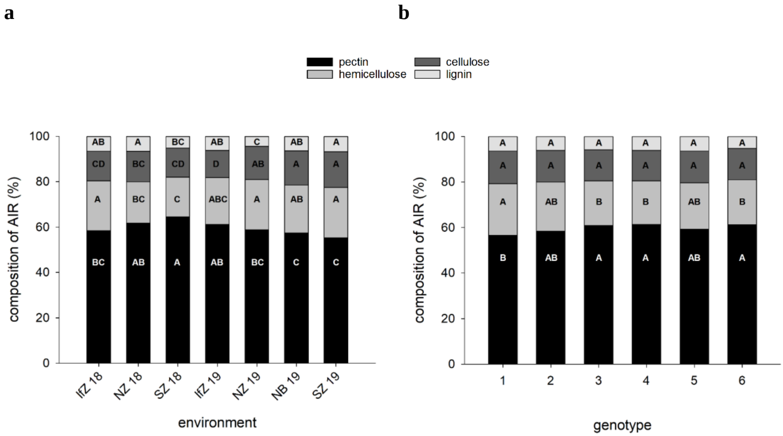

Fig. 17: Cell wall composition of sugar beet roots (AIR: alcohol insoluble residues); a: Environments as mean of 6 genotypes; (NZ 19 and NB 19 same site; NB 19 with irrigation) b: Genotypes as mean of 7 environments (Germany 2018+19); n=6. Environments or genotypes not sharing a common letter within a parameter are significantly different at α≤ .05 according to Tukey's test.

3.3. Relationship between genotypic cell wall content and tissue strength

The relationship between the genotypic AIR content and the associated puncture resistance and compressive strength is shown for each environment and additionally from the genotype screening trial with 12 genotypes in 2020 (Fig 18 a & b). At all environments, the tissue strength of the genotypes increased with increasing cell wall content (AIR).

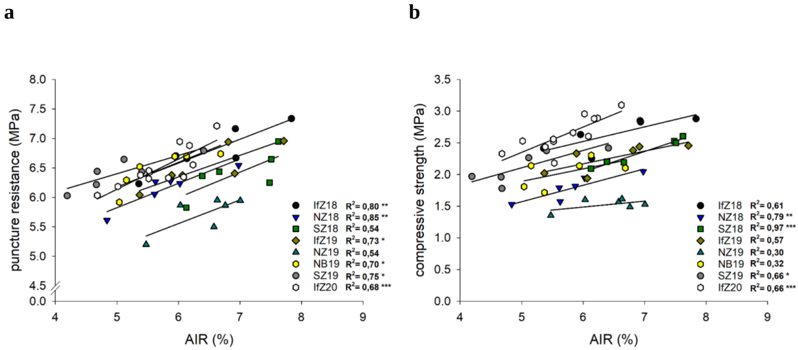

Fig. 18: Relationship between AIR content (on fresh mass basis) and puncture resistance (a) and compressive strength (b) of sugar beet roots, separately for each environment (2018+19: 6 genotypes; 2020 (white hexagon): 12 genotypes); F-Test; *= p≤ 0.05, ** p≤ 0.01, ***= p≤ 0.001.

3.4. Damage Susceptibility

The average breakage diameter of the root tip over all trials was 2.2 cm. However, genotypes and environments differed significantly in this parameter, except for the genotypes in the 2020 screening trial (Fig. 19a+b). The genotype by environment interaction was not significant in the 2018 and 2019 field trials. In environment NZ19, which had very low root tip breakage, and IfZ20, with no genotypic differences, no trend was evident between tissue strength and root tip breakage. In the other environments, root tip breakage decreased with increasing tissue strength, although this relation was not always present. A scoring of the surface damage on the same roots resulted in similar findings (data not shown).

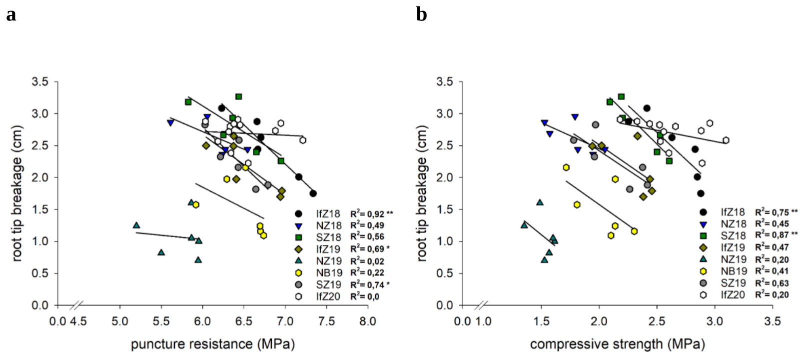

Fig. 19: Relationship between puncture resistance (a) and compressive strength (b) of sugar beet roots to root tip breakage separately for each environment (2018+19: 6 genotypes; 2020 (white hexagon): 12 genotypes). F-Test; *= p≤ 0.05, ** p≤ 0.01.

3.5. Quality and sugar loss after storage

The invert sugar accumulation of the six genotypes during storage relative to the environmental mean in the seven environments 2018 and 2019 is shown in Figure 20. Roots from five environments showed low invert sugar accumulation during storage, with less than 6 mmol kg^{-1}. In these environments genotypic differences were low. However, genotypic differences increased markedly if the environmental mean was on a higher level. The Genotypes 1, 2 and 5 showed an invert sugar accumulation above the 1:1 line and thus higher than the average.

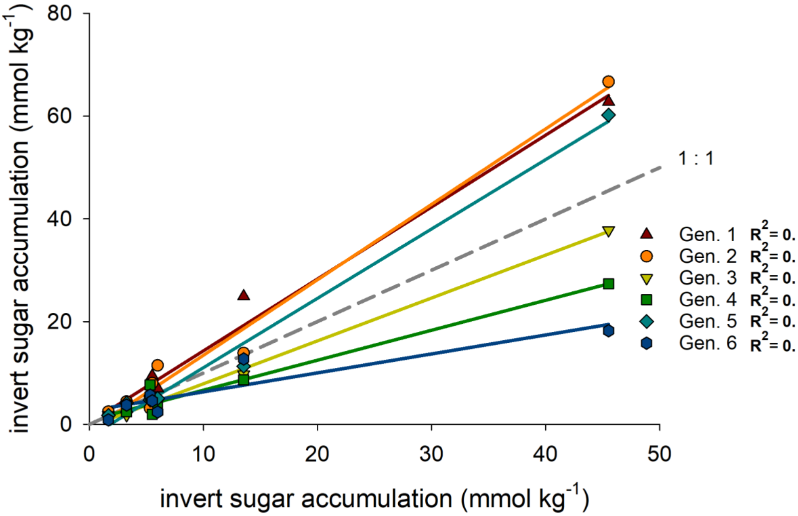

Fig. 20: Invert sugar accumulation of 6 sugar beet genotypes (y-axis) after storage in relation to the environmental mean at 7 environments in Germany in 2018 and 2019 (x-axis).

3.6. Patterns of tissue strength and storage losses

To find patterns within the 2018 and 2019 data a principal component analysis was made (Fig. 21). The data could be reduced to two principal components which described 68.6 % of the total variance. PC 1 was responsible for 53.1 % of the variance and was mainly defined by the three tissue strength parameters and the distance to rupture. The dry matter content and its fractions were also loaded on this component. PC 2 (15.5 % of the variance) was determined by the storage losses (invert sugar accumulation) and root tip breakage. Genotypes having less cell wall material and thus a weaker tissue strength tended to have higher storage losses (Fig. 21b). For the environments, no such clear pattern could be found (Fig 21a). Roots from environment SZ18 with a high tissue strength had a high invert sugar accumulation, while roots from NZ19 with a very low tissue strength had a low root tip breakage.

a **b**

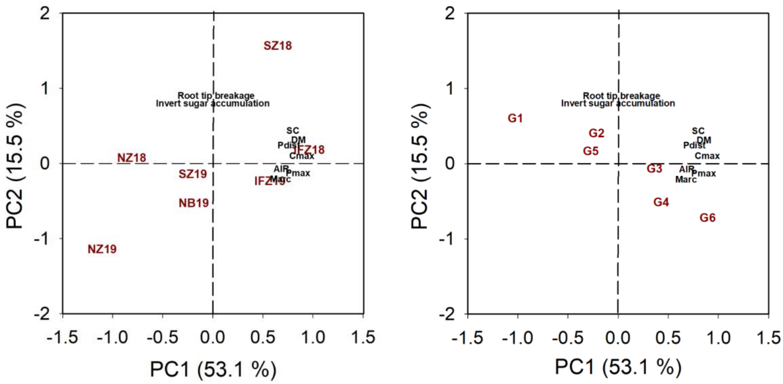

Fig. 21: Principal component analysis including tissue strength, dry matter composition and storage loss for 7 environments in Germany (2018+2019) (a) and for 6 sugar beet genotypes G1-G6 (b), bivariate plots of the 2 factors; Cdist is like Pdist and Pmean to Pmax; Pmax=Puncture resistance, Pmean= Tissue firmness, Pdist= Distance to rupture of periderm, Cmax= Compressive strength, Cdist= Distance to rupture by compression testing, DM= Dry matter, SC= Sugar content.

4. Discussion

4.1. Effect of storage on tissue strength

Environmental and genotypic variation in root tissue strength of sugar beet were described by Kleuker and Hoffmann (2021a) for freshly harvested roots. Since a high proportion of sugar beet roots are processed after a certain storage period in field clamps, the objective of this study was to analyse the effect of storage on tissue strength and to investigate the relationship between storage loss and tissue strength. Moreover, causes for differences in tissue strength were studied. Changes of tissue strength, which increase damage susceptibility of sugar beet roots after storage might lead to additional losses during transport, root handling in the sugar factory and to problems due to sugar leaching during washing (Vukov 1977). Nevertheless, information concerning the tissue strength and its behaviour during storage are scarce. The results of this study showed that storage has a significant effect on the tissue strength of sugar beet roots. The increase of puncture resistance and compressive strength was in line with findings by Nedomová et al. (2017). However, the increase in distance to rupture found by Nedomová et al. (2017) was much stronger than in this study. This might be due to different test settings, but in addition the storage effect was probably more severe.

The extent of the storage effect on tissue strength and distance to rupture differed between the environments. Minor changes in tissue strength during storage were associated with little increase in the dry matter content (water loss). Obviously, the elasticity of the periderm increased (determined as the distance to periderm rupture), when the dry matter content increased during storage, and that was dependent on the environment. If the distance to rupture is greater, the underlying tissue will be more strongly compressed before the probe ruptures the periderm. As a result, the strength of the underlying tissue (tissue firmness) may be lower. Already Vukov (1977) reported that in sugar beet the tissue elasticity is related to water loss during storage. Hatfield and Knee (1988) concluded for apples that water loss leads to maintenance of cell cohesion and that the penetrometer test is probably influenced by cell compressibility and cohesion. Abbott (2004) summarized results by Lin und Pitt (1986) that turgor pressure prestresses the cell wall and thus the cells with high turgor are more brittle and failure occurs earlier. The effect of turgor, however, is only important, if the mechanism of failure is fracturing across cells rather than cell-to-cell detachment (Harker and Hallett 1992). Since the turgor appears to influence the failure of sugar beet, fracturing across cells seems to be the mechanism of failure in this trial. In further studies it might be beneficial to inspect the rupture points by microscope.

For many fruits genotypic changes in tissue strength during storage are reported (Ahmadi-Afzadi et al. 2013). In this study, however, it was demonstrated that the genotypes responded in the same way to extented storage. Genotypes with above-average tissue strength at harvest had also an above-average tissue strength after storage. This finding is important, as it allows the conclusion that genotypic tissue strength before storage determines the strength after storage. Estimations of the genotypic influence on post-storage damage susceptibility in manufacturing and behaviour during processing are therefore possible on fresh roots. That sugar beet genotypes did not react differently to storage might be due to the fact that sugar beet do not have a ripening period with major changes in cell wall composition (Hoffmann et al. 2021). Therefore, tissue strength can be determined in fresh roots just like the other quality parameters in variety testing.

4.2. Tissue strength and cell wall

Tissue strength is probably based on the root structure. The anatomical structure of sugar beet roots was already described by Artschwager (1926a). Despite great progress in breeding this structure and the number of cambium rings have not changed significantly over the years (Loel et al. 2014). Nevertheless differences in cell size as reason for sugar accumulation and yield development between different sugar beet genotypes were discussed by Milford (1973a) and Wyse (1979). They concluded that smaller cells have a greater cell wall to cell volume ratio. Roots with small cells have less distance between the cambium rings and thus can store more sugar. However, Loel et al. (2014) stated that the relationship between cell wall content and sugar concentration is only valid for genotypes with a similar registration year. Due to breeding the dry matter allocation of sugar beet genotypes changed in the past decades towards a higher sugar content accompanied with lower cell wall content. Differences among genotypes in cell wall content (AIR, marc) indicate the genotypic tissue strength. The old genotype 6 had a significantly higher cell wall content compared to the newer genotypes, but other than Loel et al. (2014) reported, the dry matter content was also higher for the old genotype. These genotypic differences in cell wall content were reflected in the tissue strength. Kleuker and Hoffmann (2021a) described a relationship between sugar content / yield type and tissue strength. This could be confirmed as the genotypic sugar content was related to the cell wall content, which was confirmed by PCA. Also Nause et al. (2020) found a relationship between cell wall content and tissue strength, and they moreover detected differences in the cell size of sugar beet and fodder beet in microscopic analysis. However, the relationship between cell size and tissue strength is up to now not known. Thus further studies regarding sugar beet genotypes with different cell sizes and their tissue strength are needed.

Although the cell wall content of the genotypes differed significantly, there was little variation in the cell wall composition, especially in comparison to the differences in tissue strength. Minor differences in cell wall composition, with a tendency for higher pectin content in firmer genotypes was also reported by Nause et al. (2020). Pectin is found mainly in the middle lamella between the cells. Marry et al. (2006) determined that pectic polysaccharides are the main regulator for adhesion in sugar beet tissue, but calcium and ester cross-linked pectins are both required for cell adhesion. However, closer examination of the cell wall composition and cross-links of the Beta varieties did not reveal any differences in terms of tissue strength either (Schäfer et al. 2020).

For environments, the mean cell wall content of sugar beet did not reflect the mean environmental strength of the roots. This may be due to the fact that mainly sugar beet from environments with extreme drought stress differed in tissue strength from those which had not experienced drought stress. Hoffmann et al. (2005) stated that severe drought stress strongly increased the marc content of sugar beet, which is determined very early in the season. According to this the cell wall content in the NZ18 and NZ19 environments did not decrease as much as the sugar content. Thus, a low dry matter content may be an indication of low tissue strength, while a high dry matter content does not necessarily indicate higher strength.

4.3. Tissue strength and storability

High infestation with pathogens is the main reason for sugar loss and invert sugar accumulation during storage (Bugbee and Cole 1976; van Swaaij and Huijbregts 2010; Schnepel and Hoffmann 2016). Thus, genotypic differences in storage loss seem to be due to differences in pathogen infestation (Nause et al. 2020). These differences in pathogen infestation have already been linked to differences in damage susceptibility (Hoffmann and Schnepel 2016a). Thus, stronger tissue seems to reduce damage and thus entry points for pathogens, and furthermore to delay the spread of pathogens. The damage occurs mainly during harvest and the extent differs strongly between environments and thus harvest conditions. In particular, root tip breakage is strongly dependent on the harvester setting (Hoffmann 2018; Hoffmann et al. 2018a), so environments with different harvester settings might influence the root tip breakage stronger than tissue strength. Furthermore, roots from environments with drought (NZ19) are smaller and thus root tip breakage is usually smaller as well.

Genotypic differences in damage susceptibility were found in many studies (van Swaaij et al. 2003; Hoffmann and Schnepel 2016a). In our study, the compressive strength was closely negatively correlated to root rip breakage in all environments, the puncture resistance in most of the environments. Hence, the tissue strength seems to have a direct impact on root tip breakage. Since the genotypic cell wall content causes the genotypic differences in tissue strength, it confirms at the same time the negative correlation between root tip breakage and the genotypic marc content as reported by Schnepel and Hoffmann (2016) and Hoffmann et al. (2018b).

The strong environmental impact on storage loss, reported by Campbell und Klotz (2007) and Schnepel and Hoffmann (2016) was also found in this study. The temperature range of 7 to 9°C aimed to reproduce the storage conditions for sugar beet grown in temperate climates and was similar for all roots from the different environments, except SZ18 which had a shorter storage period. However, large differences in storability between genotypes were found predominantly in environments with higher level of sugar loss, as previously noted by Kenter and Hoffmann (2009). Consequently, genotypic differences can be found only in extreme environments with a high level of storage losses.

The PCA is a method to derive general effects from many different parameters. It confirmed that genotypes with a higher cell wall content and thus higher tissue strength had lower root tip breakage and less invert sugar accumulation after storage. This pattern was not evident for the environments, as roots from environment SZ18 had high tissue strength, but still high storage losses due to secondary infections. Differences in infestation are not related to the pathogen potential. Liebe and Varrelmann (2016) showed that sufficient pathogen potential is found in all environments, but not necessarily associated in all environments with a strong pathogen colonization during storage.

Although this study covered very different environments, it is still not possible to predict the level of storage losses of roots grown under certain environmental conditions with any parameter determined at harvest, the choice of a variety with a good storability gains importance for all sugar beet growing areas. From assessing 92 commercial clamps, Hoffmann (2018) concluded that unfavourable conditions and in addition a susceptible variety increase storage losses disproportionally. This emphasises the need to breed for good storability. Similar as the marc content suggested by Schnepel and Hoffmann (2016), the genotypic tissue strength can be another indirect criterion for the storability of sugar beet genotypes. In future studies it should be investigated how nondestructive methods (impedance spectroscopy, magnetic resonance imaging, hyperspectral imaging) can allow a similar and automated estimation of tissue structure and strength, damage susceptibility or storability (Opara and Pathare 2014; Metzner et al. 2014).

5. Conclusions

The root tissue strength of different sugar beet genotypes changed similarly during storage. Therefore, the impact on processes after storage can be estimated in advance with an analysis of fresh roots. For sugar beet genotypes, the pattern of interrelationships seems to be as follows: a higher cell wall content leads to a higher tissue strength, resulting in less damage at harvest, which in turn leads to lower storage losses due to reduced pathogen infestation and spread. An introduction of tissue strength as variety trait in breeding and official variety testings could thus contribute to reducing losses in future. Furthermore, the unintended development in breeding towards a lower cell wall content and tissue strength could be monitored, or even counteracted, if necessary.

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Supplementary

Supplemental Tab. 1 S1: Environmental characteristics of the field and storage trials in Germany (2018 and 2019).

Year	Site	Env.	Soil-type	Seeding date	Harvest date	Precipitation Apr-Oct.	Irrigation	Storage Duration	Storage temperature
						mm	mm	d	°C
2018	IfZ	IfZ18	Loam	19/04	15/10	245	-	82	8.1
2018	NZ	NZ18	Loamy Sand	07/04	05/10	243	90	82	8.1
2018	SZ	SZ18	Loam	07/04	04/10	276	-	55	8.6
2019	IfZ	IfZ19	Loam	08/04	14/10	334	-	84	7.5
2019	NZ	NZ19	Loamy Sand	08/04	01/10	284	-	90	7.5
2019	NZ	NB19	Loamy Sand	08/04	01/10	284	120	90	7.5
2019	SZ	SZ19	Loam	28/03	01/10	293	-	90	7.5

Supplemental Tab. 2 S2: Significant differences within texture parameters of 6 sugar beet genotypes; separated by storage treatment; 7 environments (env.) in Germany (2018 +19); n=6;
Pmax= Puncture resistance, Pmean= Tissue firmness, Pdist= Distance to rupture of periderm, Cmax= Compressive strength, Cdist= Distance to rupture by compression testing. Genotypes notsharing a common letter within a parameter are significantly different at $\alpha \leq 0.05$ according to Tukey's test.

Gen-otype	Pmax (fresh)	Pmax (stored)	Pmean (fresh)	Pmean (stored)	Pdist (fresh)	Pdist (stored)	Cmax (fresh)	Cmax (stored)	Cdist (fresh)	Cdist (stored)
1	D	D	E	D	D	BC	C	D	B	C
2	C	C	D	C	C	C	C	E	C	D
3	C	C	C	B	B	BC	A	AB	A	A
4	B	B	B	B	AB	A	A	B	A	AB
5	C	C	D	C	B	AB	B	C	B	C
6	A	A	A	A	A	AB	A	A	AB	B

Supplemental Tab. 3 S3: Significant differences within quality parameters of 6 sugar beet genotypes; separated by storage treatment; 7 environments (env.) in Germany (2018 +19); n=6;
DM= Dry matter, SC= Sugar content, AIR= Alcohol insoluble residue, Marc= hot water insoluble residue. Genotypes not sharing a common letter within a parameter are significantly different at α≤0.05 according to Tukey's test.

Genotype	DM (fresh)	DM (stored)	SC (fresh)	SC (stored)	AIR (fresh)	Marc (fresh)
1	E	E	D	D	E	D
2	B	BC	A	A	C	C
3	B	B	B	A	B	B
4	C	C	C	B	B	B
5	D	D	D	C	D	C
6	A	A	AB	A	A	A

Manuskript IV:

Root tissue strength and storage losses of sugar beet varieties as affected by N application and irrigation4

Christa M. Hoffmann, Gunnar Kleuker, André Wauters, William English, Martijn Leijdekkers

Abstract

There is some evidence that sugar beet root tissue strength affects damage susceptibility and storage losses. This study aimed at analyzing the effect of N application and of irrigation on tissue strength of sugar beet varieties, on root composition, and on root tip breakage and storage losses. For this purpose, field trials in six replicates with three sugar beet varieties were carried out with three N doses in The Netherlands and Belgium in 2018 and 2019, alternatively with three irrigation treatments in Sweden 2018 and 2019. Results show a low impact of N application and irrigation on puncture resistance, tissue firmness and compressive strength of the roots, while varieties differed always stronger and significantly. Cell wall composition (pectin, hemicelluloses, celluloses, lignin) did not differ markedly in roots from different environments (sites, years) and varieties, giving no explanation for differences in tissue strength. However, the percentage of cell wall material (AIR, marc) and of dry matter were higher in roots with higher tissue strength. Root tip breakage and sugar losses during storage tended to be lower when root compressive strength of varieties was higher. Hence, root tissue strength could serve as an indirect selection criterion for reduced damage susceptibility and improved storability of sugar beet varieties.

Keywords: Puncture resistance, Compressive strength, Tissue firmness, Storability, Root tip breakage, Sugar loss

[4]C. M. HOFFMANN; KLEUKER, G.; WAUTERS, A; ENGLISH, W; LEIJDEKKERS, M. (2022): Root tissue strength and storage losses of sugar beet varieties as affected by N application and irrigation. In: Sugar Ind. 147, 34-41. https://doi.org/10.36961/si28254.

1. Introduction

Sugar beet roots are exposed to multiple mechanical stresses during harvesting and processing (Steensen 2002a; Smed et al. 1996). Mechanical strain causes losses of whole root parts in the field and the factory yard, but leads also to more inconspicuous injuries, such as abrasions and bruises (Wiltshire and Cobb 2000a; Brown et al. 2002). In consequence, damages at harvest increase sugar losses during storage (Hoffmann and Schnepel 2016b; Schnepel and Hoffmann 2016; Steensen and Augustinussen 2002), as the wound healing process requires energy and moreover, injuries offer entry points for pathogen infestation (Akeson 1978; Campbell and Klotz 2006a). Recent studies suggests that damage susceptibility and storage losses of sugar beet roots will be lower if the tissue strength is higher (Kleuker and Hoffmann 2020; Nause et al. 2020). The tissue strength of sugar beet roots is influenced by the genotype, and furthermore, by the environmental conditions under which the crop grows (Kleuker and Hoffmann 2021). It is assumed that these impacts are attributable to the changes in the composition of the root, which then alters the mechanical behaviour and thus the resistance to damage.

The most important environmental conditions concerning crop growth are weather and soil conditions. Among the conditions that can be influenced during cultivation, N application has the greatest effect on sugar beet yield, but also on the composition of the root. Increasing N supply usually alters the composition as more water and non-sugar substances are stored with increasing cell size, resulting in a reduced dry matter content (Milford and Watson 1971; Hoffmann 2005).

Another factor altering root composition is water availability. Severe drought stress was demonstrated to affect tissue strength of sugar beet roots considerably (Kleuker and Hoffmann 2021). Usually, irrigation leads to a dilution of all soluble cell compounds resulting in a lower dry matter content. With increasing cell size due to water uptake, the cell walls as structural components get a lower proportion of the root fresh matter. It is therefore assumed that an altered composition can affect the firmness of the root, causing a lower tissue strength with high N supply and with irrigation.

Kleuker and Hoffmann (2020) and (2021) reported significant differences in the tissue strength of sugar beet varieties. Varieties with higher tissue strength tended to have lower root tip breakage and lower sugar losses during storage (Kleuker and Hoffmann 2022). These effects could not be found for roots from all locations, but only under conditions (in environments) that resulted in high sugar losses occurring in general. It is not yet clear how changes in root composition and texture due to N supply or irrigation may influence root tip breakage and storage losses.

The objective of the study was to analyse the effect of N supply and of irrigation on tissue strength and composition of roots of different sugar beet varieties, and furthermore, to find out if differences in tissue strength affect root tip breakage and storage losses.

2. Material and Methods

2.1. Field trials

In 2018 and 2019 field trials with different N application were conducted at a site in Belgium (IRBAB: 2018 in Hannut, 2019 in Lens) and a site in the Netherlands (IRS: 2018 in Colijnsplaat, 2019 in Lelystad) as block design with six replicates. Two factors were varied: three sugar beet varieties with different yield type were included, furthermore, N fertilizer application was varied with a control treatment (N_{min}: mineral N in soil in spring), a treatment according to the regional advice (N_{adv}: around 120 kg N ha^{-1}) and a treatment with an extra 80 kg N ha^{-1} in addition to the N adv treatment (N_{adv}+80).

In Sweden field trials with the same three varieties in three irrigation treatments (No irrigation, optimal irrigation, heavy irrigation before harvest) were carried out in 2018 and 2019 (NBR: Löddeköpinge) (Tab. 6).

Tab. 6: Water supply of plots with growing season precipitation and irrigation, Sweden (NBR) 2018 and 2019. Number of irrigations shown in brackets.

growing season	No irrigation (mm)	Optimal irrigation (mm)	Irrigation before harvest (mm)
24.04. to 15.10.2018	152	152 + 135 (5)	152 + 60 (2)
10.04. to 24.10.2019	359	359 + 118 (4)	359 + 35 (1)

All field trials were sown beginning of April, dependent on soil and weather conditions. They were run according to the national guidelines and were kept as free as possible of weeds, pests, and diseases.

2.2. Storage experiments

In October and November 2018 and 2019 plots were machine harvested. Roots were sent to Göttingen (IfZ). The 80 to 100 roots per plot were randomly divided into reference (prior to storage) and storage samples according the description from the IIRB (Legrand et al. 2016).

Prior to storage the root tip breakage was determined at ten roots per plot, measuring the average diameter at the root tip with a ruler.

The storage sample consisted of two air-permeable bags, which were separately weighed (weight before storage). Samples were stored in climate containers at constant temperature. The samples were covered with an additional layer of beets to reduce possible position effects through differences in transpiration. The average temperature was 8.6 °C in 2018 and 8.3 °C in 2019 with a relative humidity of 99%. Roots were stored for 70 days in 2018, and for 74 days (IRS, NBR) and 50 days (IRBAB) in 2019. After storage, samples were weighed and the difference to the weight before storage was calculated.

2.3. Texture measurements

Puncture and compression tests were performed with five beets per plot with a Texture Analyser with a 100 kg load cell (TA.XTplus 100, Stable Micro Systems, Godalming, UK) as described in detail by Kleuker and Hoffmann (2019). Sugar beet roots were washed and allowed to dry before measurement.

Puncture tests were conducted with a cylindrical probe (Ø= 2 mm) at three measurement points per root around the biggest root diameter. Root groove and crown were omitted. The measurement determines the puncture resistance as force to penetrate the periderm and the tissue firmness as average resistance of the underlying tissue (until 5 mm depth).

Compression tests were conducted with the same roots. A root slice of at least 20 mm height was cut at the biggest root diameter. From the slice, two cylindrical samples (near the centre, more to the outside) with a diameter of 18 mm were cut with a cork borer and trimmed to a height of 20 mm. Results show the compressive strength as mean value of the two cylinders.

All parameters describing root texture (puncture resistance, tissue firmness, compressive strength) are summarized as tissue strength.

2.4. Analysis

Beet quality was determined for the reference samples and the stored samples. A homogeneous brei sample was produced, shock frozen and stored at -20 °C until analysis. For the analysis of sugar, potassium, sodium, and amino-N (blue number method) brei was clarified with 0.3 % Al-sulphate solution. The analysis was conducted with an automated beet laboratory system (Anton Paar Opto-Tex GmbH, Seelze, Germany) according to routine methods as described by ICUMSA (1994, 2007a, 2007b). Glucose content was determined using an immobilized enzyme biosensor (Firma Dr. Müller, Freital, Deutschland); (ICUMSA 2019).The invert sugar content was calculated by multiplication of the glucose content with the factor of 1.735 (Vermeulen 2015).

The DM content was determined after drying at 105 °C until constant mass. Alcohol insoluble residues (AIR) describes the alcohol insoluble components in the sugar beet root that remain after two times extraction of the beet brei with 95% ethanol (McFeeters and Armstrong 1984; Sila et al. 2005). AIR was dried at 105 °C for 24 hours to constant mass and is expressed as percentage of root fresh matter. From AIR the cell wall composition was determined according to van Soest et al. (1991). Cell wall components, soluble and insoluble pectin, hemicellulose, cellulose, and lignin were quantified by the solubility in different detergents. The cell wall components are expressed as percentage of AIR. The marc content includes all components, which remain after four times extraction with water and lastly acetone and is used in the sugar industry to estimate the pulp content of beets (Reinefeld and Schneider 1983).

2.5. Calculation of storage loss

The relative sugar loss during storage was calculated as difference between the amount of sugar before and after storage, which was set in relation to the initial amount of sugar and was referred to thermal time (°Cd; accumulated mean daily temperature) to compare roots with different storage periods. The amount of sugar before storage was calculated from the weight of the storage sample before storage, and soil tare and sugar content of the reference. The amount of sugar after storage was calculated from the weight of the stored sample, the soil tare, and the sugar content after storage. The percentage soil tare was calculated for both the reference and storage samples as weight difference between washed and unwashed roots in relation to the washed roots.

2.6. Statistics

Data were checked for normal distribution and homogeneity of variance (Kozak and Piepho 2018). Sites and years were summarized as environments for the ANOVA. The analysis was run with the SAS Desktop-Version 9.4 (SAS Institute, Inc., Cary, NC, USA) using PROC MIXED followed by a Post Hoc Tukey Test on plot means. Variance components were estimated with the PROC VARCOMP function using REML method. Significant effects are indicated with *, ** or *** for $p \leq 0.05$, 0.01 or 0.001, while ns. is not significant. Different letters indicate significant differences among varieties for a given treatment.

3. Results

The impact of N supply on sugar beet root texture parameters is shown in Fig. 22 A-C. Increasing N supply significantly affected puncture resistance (Fig. 22 A), tissue firmness (Fig. 22 B) and compressive strength (Fig. 22 C), but the effect was rather low. By contrast, variety had a substantial impact on tissue strength. Variety 3 had the highest values for tissue firmness, compressive strength, and for puncture resistance, although the latter was not significantly different to variety 2. Variety 2 had the lowest compressive strength, but significantly higher puncture resistance and tissue firmness than variety 1. There was no significant interaction between N supply and variety for puncture resistance and tissue firmness (Tab. 7).

Root tip breakage before storage was not affected by N supply (Fig. 22 D). Variety 3 had a significantly lower root tip breakage than variety 1 and 2, which did not differ. The ranking of varieties was similar for the invert sugar content and the sugar loss after storage (Fig. 22 E, F), while the N supply affected only the invert sugar content after storage.

The dry matter (DM) and AIR content of the roots was significantly affected by N supply (Fig. 22 G-H). However, the effect of varieties on composition was much more pronounced (Fig. 22 I). Variety 1 had a considerably lower dry matter content compared to variety 2 and 3, which did not differ. All varieties differed significantly in their AIR and marc content with variety 3 featuring the highest, variety 1 the lowest values. There was no interaction between N supply and variety.

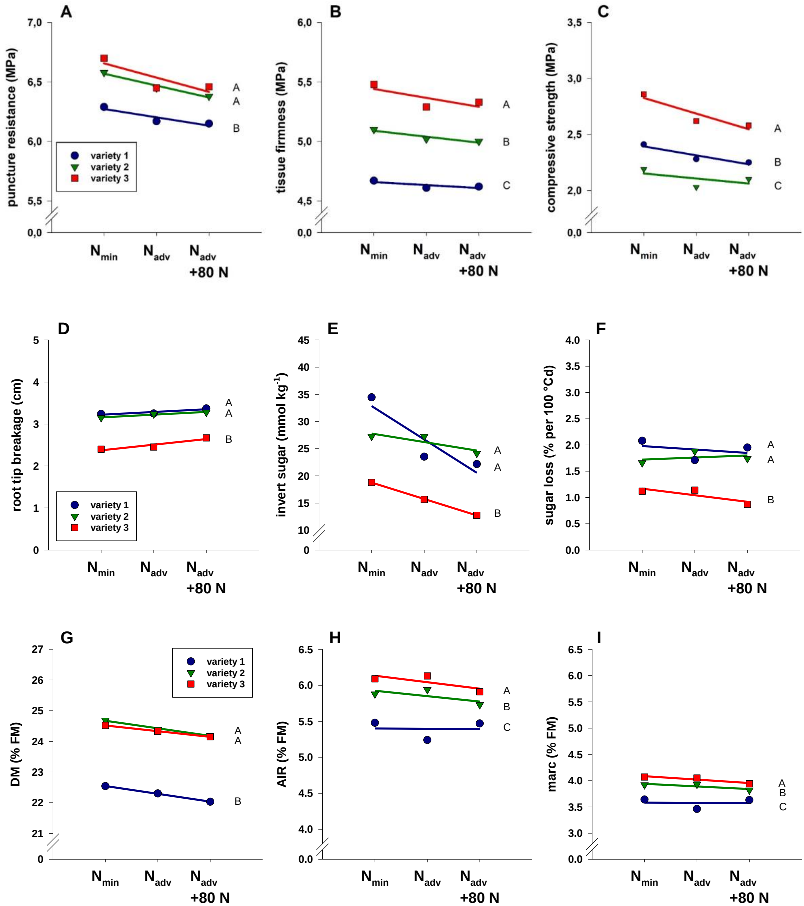

Fig. 22: Effect of N application on root texture (A, B, C), storage (D, E, F) and root composition (G, H, I) of three sugar beet varieties, mean of sites in Belgium (IRBAB) and the Netherlands (IRS) in 2018 and 2019. Sugar loss: 100% = sugar mass before storage, DM = dry matter, AIR = alcohol insoluble residues (cell wall material), marc = water insoluble residues. Different letters indicate significant differences between varieties as mean of the treatments.

Tab. 7: Variance components of texture parameters, root tip breakage, invert sugar after storage and sugar loss during storage of sugar beet; three varieties in four environments (site x year) in Belgium (IRBAB) and the Netherland (IRS) in 2018 and 2019.

% of total Variance										
	Environment (E)	**Variety (Var)**	**Fertilizer (F)**	**E x Var**	**E x F**	**Var x F**	**E x Var x F**	**Block (E)**	**Block (E x F)**	**Error**
Puncture resistance (MPa)	47.0	16.3	4.1	3.2	5.1	0.0	1.1	0.0.	0.8	22.4
Tissue firmness (MPa)	14.7	64.1	0.9	3.6	2.0	0.0	1.6	0.5	0.0	12.6
Compressive strength (MPa)	6.1	63.8	6.8	4.6	0.6	1.3	0.7	0.0	0.0	16.0
Root tip Breakage (cm)	14.8	30.9	0.7	0.6	0.0	0.0	0.0	0.0	2.1	50.9
Invert sugar mmol kgFM^{-1}	42.3	11.4	3.2	9.8	3.0	2.7	0.0	2.1	1.2	24.3
Sugar loss per 100 °Cd (%)	16.7	25.0	0.0	8.5	1.5	1.5	0.0	2.7	1	43.1

Puncture resistance and tissue firmness were significantly affected by irrigation, with irrigated beets being slightly stronger. Compressive strength was not affected by the irrigation treatment (Fig. 23 A-C). Variety effects were similar as in the N treatments: variety 1 had a significantly lower puncture resistance than variety 2 and 3, while for tissue firmness variety 2 and 3 differed significantly. In compressive strength, variety 2 showed the lowest value, variety 3 the highest. There was no interaction between irrigation and variety.

Irrigation had no effect on root tip breakage, whereas variety effects occurred with variety 3 obtaining the lowest root tip breakage (Fig. 23D). For the invert sugar content and sugar loss after storage, highest values occurred for the irrigated crops (Fig. 23 E, F), whereas there was no difference between no irrigation and irrigation before harvest. Variety 3 had the lowest invert sugar content and sugar loss irrespective of irrigation, while variety 1 and 2 showed higher values.

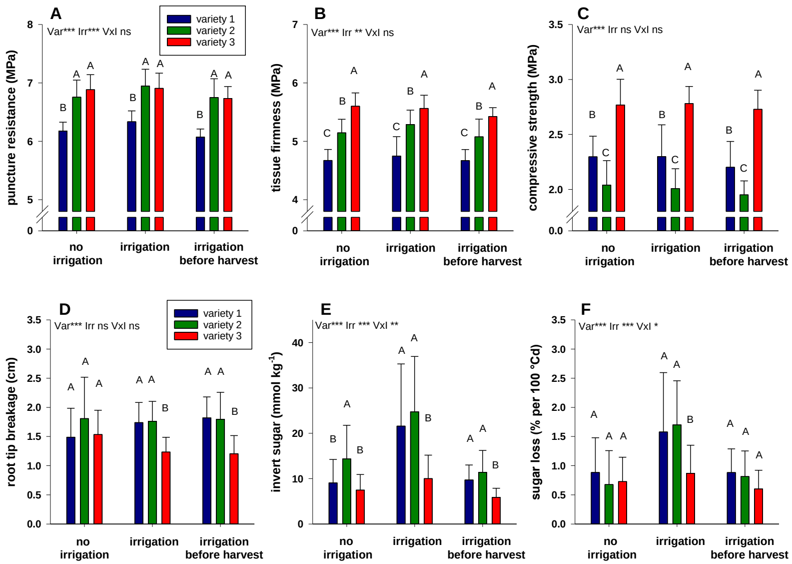

Fig. 23: Effect of irrigation on root texture (A, B, C) and storage (D, E, F) of three sugar beet varieties, Sweden (NBR); mean of sites in 2018 and 2019. Sugar loss: 100% = sugar mass before storage. Different letters indicate significant differences between varieties for the treatments.

The composition of AIR with the individual cell wall components is shown in Fig. 24. A major part of cell wall components was pectin with around 60% of the AIR, followed by hemicelluloses (20%), celluloses (15%) and lignin (5%). In the composition of the cell walls, there was neither much difference between the environments (Fig. 24 A) nor between the varieties (Fig. 24 B), although the percentage of AIR differed.

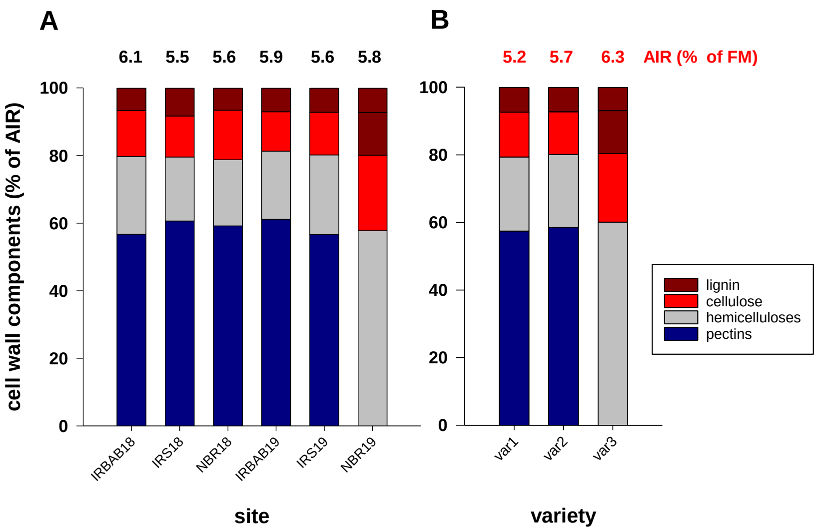

Fig. 24: Alcohol insoluble cell wall material (AIR) content and cell wall composition of sugar beet at different sites (A) and for different varieties (B); sites: mean of three varieties with three treatments (IRBAB: N application, IRS: N application, NBR: irrigation); varieties: mean of six sites with three treatments.

The relation of root tip breakage and storage losses of the three varieties to their compressive strength in the trial years is presented in Fig. 25 A, C, E and at the six trial sites (Fig. 25 B, D, F). For the varieties, it is obvious that a higher compressive strength of the root resulted in lower root tip breakage, lower invert sugar content after storage and lower sugar losses. Only if root tip breakage, invert sugar content and sugar loss were at a generally very low level, then the compressive strength of the root had a lower impact.

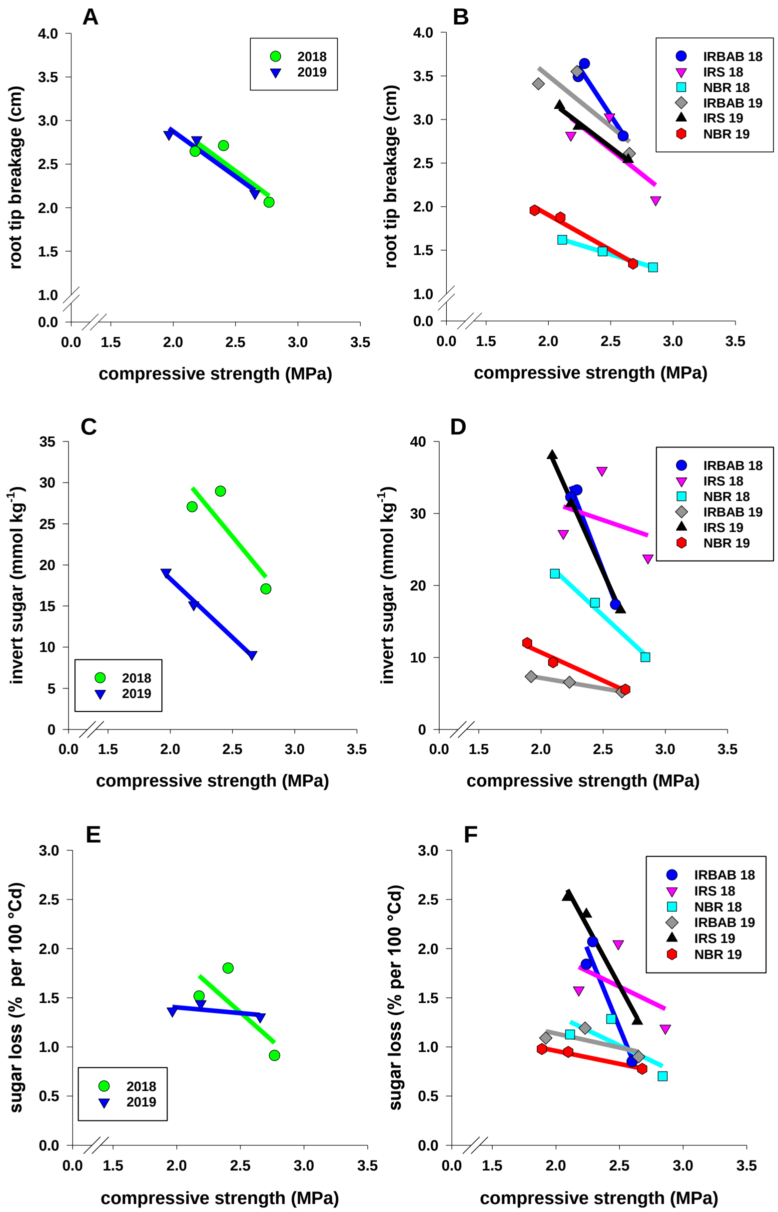

Fig. 25: Relationship between root tip breakage (A, B), invert sugar content after storage (C, D) and sugar loss during storage (E, F) and compressive strength of roots of three sugar beet varieties for years (A, C, E) as mean of three sites with three treatments (N application or irrigation) per year, and for sites (B, D, F) as mean of three treatments. Sugar loss: 100% = sugar mass before storage.

4. Discussion

First studies have demonstrated a high genotype effect with low genotype by environment interaction for texture parameters of sugar beet roots (Kleuker and Hoffmann 2020, 2021). In these studies, all crops were cultivated at different sites using standardized cultivation methods. The effect of increasing N application and different irrigation treatments was now studied under the assumption that these are the management operations which exert the dominant impact on the composition of the sugar beet root, and therefore possibly may affect root tissue strength.

4.1. N effect

Increasing N supply usually alters the composition of the root with increasing cell size. The dry matter content is reduced with high N application, as it tended to be in our study. But as the dry matter of sugar beet mainly consists of sugar (ca. 74%, Hoffmann 2010b), the impact of N application on the cell wall components determined as AIR and marc was very low. This confirms results by Schäfer et al. (2020) with different Beta varieties, where increasing N supply with excess N doses did not alter root composition significantly. N application had no effect on monomer composition of polysaccharides, degree of acetylation and methylation of pectin, and on cell wall bound phenolic components (ferulic acids) (Schäfer et al. 2020).

Due to this small impact of N application on tissue composition, it was not surprising that the effects on root tissue strength were also low, even if different to expectations. Consistently, for increasing N supply neither a clear effect on root tissue strength, root tip breakage and storage losses, nor on the composition of the structural components of the storage root was detected.

4.2. Irrigation effect

It was expected that extra water supply, in particular given shortly before harvest, would make the root tissue weaker. However, irrigation did not have a strong effect on tissue strength.

From the reduction in root weight (data not shown) it can be deduced that the treatment not receiving irrigation water experienced drought stress. This would have been only very moderate in 2019, but in 2018 it would have been similar to the severe stress described in Kleuker and Hoffmann (2021). In their study, severe drought stress resulted in very low tissue strength associated with high storage losses as compared to sites with better water supply. This effect is not seen in the current study. Further, irrigation did not alter the dry matter content and composition of the roots (data not shown), pointing to a minor impact on the water content.

A possible explanation for the greater storage loss for the irrigated roots independent of tissue strength is disease load. Perhaps the water supply under irrigation was more than optimal, so that additional factors such as certain diseases could arise. In 2018 the roots under irrigation in Sweden suffered from a severe infestation of chronic *Aphanomyces*. *Aphanomyces* has been shown to reduce the storability and severely enhance sugar losses during storage (Campbell and Klotz 2006b). It seems likely this infestation would have been more severe in the irrigated treatment owing to the wetter microclimate during the months of the growing season when temperatures are optimal for infections. The excessive supply (in relation to the crop demand) in combination with pathogen infections is most likely the reason for the adverse effect of irrigation on storability, which was not related to root strength. In their response on irrigation, significant differences between the varieties occurred indicating genotypic differences in the susceptibility.

4.3. Variety effect

The results confirm the substantial effect of the variety for root tissue strength as also reported by Kleuker and Hoffmann (2020, 2021). This can probably be explained by the fact that the structure of internal tissue (cambium rings, cell number) in the root of different sugar beet types is established very early (Artschwager 1926b; Milford 1973). All other yield and quality traits start to develop gradually during the growing period, so that their development is much more prone to environmental changes during the season.

The composition of the cell walls turned out to be rather similar among varieties, as also reported by Kleuker and Hoffmann 2022. Schäfer et al. (2020) carried out extensive analyses of cell wall components of Beta varieties. In their study even single cell wall polysaccharides, their cross-linkage and cell wall bound phenolic compounds were not related to the pronounced differences in tissue strength of Beta varieties. The cell wall composition of the four Beta varieties (two distinct sugar beet varieties, fodder beet, garden beet) was surprisingly similar. Therefore, the authors suggested that compositional differences of the cell wall do not provide convincing explanations for differences in root strength. The most pronounced difference also in the current trial was the dry matter content and the AIR content in fresh matter of the varieties. This suggests that the cell size with the respective difference in water content might play a decisive role for their strength. Nause et al. (2020) found first indications that cell sizes differ in varieties with different root strength. For two varieties with different storability Madritsch et al. (2020) showed differences in the number of parenchyma cells, cell area and periderm thickness. However, it has to be tested in further experiments with more varieties, if this is a causal relation and if the number of cambium rings with the higher percentage of vascular tissue and/or the cell size and periderm thickness are related to the root tissue strength of sugar beet varieties.

4.4. Root tissue strength and storability

Root tissue strength was analyzed not only as an interesting new trait of sugar beet, but primarily with the aim to identify the relation to harvest damage and storage losses. Results show that root tip breakage, invert sugar content after storage and sugar losses of varieties were closely related to compressive strength, when storage losses had reached a certain level. As there is a generally close correlation between the three texture parameters (Kleuker and Hoffmann 2021), damage susceptibility and storage losses are also related to puncture resistance and tissue firmness. Varieties with a higher tissue strength (puncture resistance, tissue firmness, compressive strength) had lower root tip breakage losses, less damage, hence lower pathogen infestation during storage and finally tended to have lower storage losses as also reported by Kleuker and Hoffmann (2022). However, even if the variety ranking did not change between sites, the absolute root tissue strength and storage losses did. It is therefore only possible to compare the absolute tissue strength of varieties grown under the same conditions, and not to compare varieties from different sites (fields) or to estimate absolute storage losses from the tissue strength.

Despite this constraint, the determination of tissue strength could be an interesting trait to approach the damage susceptibility and storability of sugar beet varieties. Previously the marc content was suggested as a trait also related to storability (Schnepel and Hoffmann 2014, 2016), which could be regarded as an indirect criterion and is closely related to root texture. As the tissue strength has no interaction between environments and genotypes (Kleuker and Hoffmann 2021), only few trial sites will be needed to get a good estimate of the root tissue strength of different genotypes.

5. Conclusions

Surprisingly, root tissue strength of sugar beet was much more influenced by the variety than by the most important treatments usually affecting root composition, N supply and water availability. It can therefore be assumed that other agronomic treatments will most likely not alter root texture either. An exception could be diseases, in particular root rots such as rhizoctonia, but probably also infection with virus yellow (Hossain et al. 2021) and SBR (Syndrome basses richesses; Pfitzer et al. 2020), which will most likely lead to a reduction of root tissue strength because of rotten tissue and a general disorder of root metabolism. The effect of irrigation on storage losses was independent of tissue strength; underlying causes should thus be further investigated.

Root tissue strength of varieties turned out to be a major factor influencing root tip breakage, invert sugar content after storage and sugar losses. Kleuker and Hoffmann (2021) showed also in commercial sugar beet varieties surprisingly high variation in root tissue strength, indicating a potentially large effect on the functionality of roots. Because of the close correlation, tissue strength could serve as an indirect selection criterion for reduced damage susceptibility and improved storability of sugar beet varieties. It has to be evaluated in which relation high root tissue strength is to other breeding targets, in particular to the performance of varieties. Kleuker and Hoffmann (2021) found a negative correlation for varieties between root yield and tissue strength, so that there could be tradeoffs. Furthermore, a target value concerning root strength for the factory processing quality must be discussed as well, as that could additionally differ from demands for beet cultivation and storage.

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5 Epilog

Die Grundlage für eine effiziente Verarbeitung von Zuckerrüben ist ein hoher Zuckergehalt bei gleichzeitig geringen Anteilen an Nichtzuckerstoffen. Während der Lagerung von Zuckerrüben wird sowohl durch den Stoffwechsel der Zuckerrübe als auch durch Wundheilungsprozesse nach Beschädigungen und durch Pathogenbefall Zucker abgebaut und qualitätsmindernder Invertzucker angereichert. Die Festigkeit der Zuckerrübe könnte ein Merkmal sein, um die Beschädigungsempfindlichkeit und Lagerfähigkeit abzuschätzen. Im Folgenden werden die physiologischen Grundlagen der Festigkeit von Zuckerrüben erläutert. Des Weiteren wird die Auswirkung unterschiedlicher Festigkeiten auf die Beschädigungsempfindlichkeit und Lagerfähigkeit erörtert.

5.1 Festigkeitsmessung an Zuckerrüben

Die bis zum Zeitpunkt dieser Arbeit veröffentlichten Studien zur Texturmessung von Zuckerrüben waren in ihrer Durchführung nicht einheitlich und teilweise in der Methodenbeschreibung ungenügend.

In Manuskript I wurden vielfältige Einflussfaktoren untersucht, um daraus Empfehlungen für die Messung der Festigkeit von Zuckerrüben mit einem Texture-Analyser abzuleiten. Die Empfehlung, Zuckerrüben vor dem Messen vorsichtig zu waschen, wurde bereits in den meisten vorherigen Studien umgesetzt (Gemtos 1999; Gorzelany und Puchalski 2000; Senge und Hajinezhad 2009), jedoch ohne vorherige Überprüfung des Effektes auf die Messung und somit ohne Kenntnis über den Einfluss auf das Messergebnis.

Die bei Gemtos (1999) und Senge und Hajinezhad (2009) für den Kompressionstest und bei Gorzelany und Puchalski (2000) für den Penetrationstest gefundenen Unterschiede zwischen den einzelnen Messorten in der Rübe wurden bestätigt. Wie bereits für andere Pflanzenarten wie Kartoffeln (Anzaldúa-Morales et al. 1992; Koch et al. 2019) bestimmt wurde, muss die Messung immer an einer ähnlichen Stelle des Rübenkörpers durchgeführt werden, damit die Ergebnisse vergleichbar sind.

Die Probenanzahl beeinflusst maßgeblich die erforderliche Arbeitszeit pro Versuch und ist somit für die Versuchsplanung eine wichtige Kenngröße. In den vorherigen Untersuchungen gab es große Unterschiede in der Anzahl an Messungen pro Rübe und in der Anzahl an untersuchten Rüben. Zur Bestimmung von Sortenunterschieden bei Zuckerrüben sollten mindestens sechs Rüben pro Sorte mit jeweils einer Messung analysiert werden. Bei inhomogenen Beständen sollte eher die Anzahl an Rüben statt der Anzahl an Messungen pro Rübe erhöht werden, um den Bestand besser abzubilden.

Daher ist es wichtig, vor der Ernte die Homogenität der Bestände zu überprüfen, um bei Versuchen mit mehreren Standorten die Erntetermine an den Durchsatz bei der Messung anzupassen und die Lagerungsperiode zwischen Ernte und Messung zu begrenzen.

Durch die exakte Beschreibung einer definierten Methode war es möglich, in den folgenden Untersuchungen (Manuskript II-IV) und in den Untersuchungen von Nause et al. (2020) und Nause et al.

(2021) Umwelt- und Sortenunterschiede reproduzierbar zu erfassen. Alle Untersuchungen zeigten auch eine hohe Korrelation zwischen den beiden Penetrationsparametern (Penetrationswiderstand und Gewebefestigkeit) (Kleuker und Hoffmann 2020; Nause et al. 2021). Somit wäre es möglich, die Messung auf nur einen der beiden Parameter zu reduzieren. Der Penetrationswiderstand ist dabei vorzuziehen, da dieser die Kraft darstellt, die erforderlich ist, um das Periderm der Rübe zu durchdringen. Somit spiegelt der Penetrationswiderstand die Kraft wider, welche erforderlich ist, um die Rübe als Eintrittspforte für Krankheitserreger bei Lagerung zu beschädigen (Nause et al. 2021). Zudem kann die Penetrationsmessung auch mit Hilfe eines Handmessgerätes durchgeführt werden (English et al. 2022). Erste Ergebnisse zeigten, dass es damit möglich ist, genotypische Unterschiede bereits während der Vegetationsperiode zu erfassen. Jedoch kann bei dieser Messung der Einfluss des Bedieners größer sein als der Unterschied zwischen Sorten, so dass eventuell nur größere Unterschiede in der Festigkeit festgestellt werden können.

5.2 Einfluss der Umwelt und agronomischer Maßnahmen auf die Festigkeit von Zuckerrüben

Die Festigkeit von Zuckerrüben wurde von der Umwelt signifikant beeinflusst, allerdings ist der Einfluss erheblich geringer als auf den Rübenertrag und die Qualitätsparameter. Der Einfluss der Umwelt auf die Festigkeit zeigte sich insbesondere in Umwelten mit starkem Trockenstress während der Vegetationsperiode (Manuskript II), in denen die Festigkeit deutlich verringert war. Dies galt insbesondere für Umwelten, in denen die Zuckerrüben durch einen geringen Rübenertrag und Zuckergehalt durch Trockenstress gekennzeichnet waren.

Trockenstress beeinträchtigt den Rübenertrag hauptsächlich durch eine verringerte Zellexpansion (Hoffmann 2010), daher reduziert Trockenstress den Zellwandgehalt nur geringfügig. Dies wird dadurch verstärkt, dass der Zellwandgehalt bei Zuckerrüben bereits mit der Kambiumringbildung in einem sehr frühen Wachstumsstadium festgelegt wird (Hoffmann et al. 2005). Somit konnte aus dem umweltspezifischen Zellwandgehalt kein Rückschluss auf die Festigkeit der Rübe gezogen werden. Der Zusammenhang zwischen Festigkeit und Zuckergehalt hingegen war deutlicher zu erkennen. Da die Trockenmasse zum Großteil aus Zucker besteht und der Zellwandgehalt einen geringen Anteil ausmacht, sorgt eine Reduktion des Zuckergehaltes durch Trockenstress für eine starke Veränderung des Trockenmassegehaltes (Hoffmann et al. 2005). Somit könnte ein geringer Trockenmassegehalt zur Ernte ein Indikator für eine niedrige Festigkeit der Rübe sein. Allerdings deutete umgekehrt ein hoher Trockenmassegehalt nicht notwendigerweise auf eine hohe Festigkeit hin.

Die Auswirkungen agronomischer Maßnahmen waren entgegen der Erwartung gering. Stickstoffdüngung und Wasserverfügbarkeit haben einen deutlichen Effekt auf den Ertrag von Zuckerrüben (Hoffmann et al. 2021), sodass erwartet wurde, dass ebenfalls die Festigkeit beeinflusst wird. Der Effekt der Stickstoffdüngung durch vergrößerte Zellen (Milford und Watson 1971) war jedoch erst bei deutlich über der regionalen Empfehlung gedüngten Zuckerrüben signifikant. Auch Drath et al.

(1984) fanden erst bei sehr hoher Stickstoffdüngung einen negativen Einfluss von Stickstoff auf die mechanischen Eigenschaften von Zuckerrüben. Somit lässt sich die Vermutung von Wiltshire und Cobb (2000), dass die Zuckerrüben durch stickstoffbedingtes Zellwachstum weicher werden, nur bedingt bestätigen.

Der Einfluss der Beregnung auf die Festigkeit war in den hier dargestellten Versuchen (Manuskript II und IV) nicht einheitlich. Während im Manuskript II das zusätzliche Wasserangebot die Festigkeit der Zuckerrüben unter Trockenstress erhöhte, konnte in Manuskript IV nicht dieser starke Effekt nachgewiesen werden. Auch eine Bewässerung kurz vor der Ernte hatte nur eine geringe Auswirkung auf die Festigkeit der Zuckerrüben. Trotz ähnlicher Witterungsbedingungen im Jahr 2018 in Schweden (Manuskript IV), waren die Auswirkungen von Trockenstress auf die Qualitätsparameter deutlich geringer als in den Umwelten NZ18 und NZ19 in Manuskript II. Somit scheint der Effekt einer Beregnung auf die Festigkeit unter normalen Wachstumsbedingungen gering zu sein, bei starkem Trockenstress dagegen eine höhere Festigkeit zu fördern.

Im Rahmen dieser Arbeit konnte aufgrund der nicht einheitlichen Beschädigung durch unterschiedliche Roder und Rodereinstellungen in den einzelnen Umwelten kein Zusammenhang zwischen der umweltspezifischen Festigkeit und dem Ausmaß der Beschädigung der Rübe gefunden werden. Wenn weichere Rüben eine höhere Beschädigungsempfindlichkeit aufweisen, dann müssten gerade Rüben von Standorten mit starkem Trockenstress vorsichtig gerodet werden. In der Praxis verfolgt die geringe Geschwindigkeit beim Roden auf Trockenstressstandorten zumeist das Ziel, die Verluste an kleinen Rüben gering zu halten, hätte aber darüber hinaus den Vorteil, die Beschädigung der Rüben mit geringerer Festigkeit zu vermindern. Zudem ist der Erdanhang meist geringer, sodass die Reinigung weniger aggressiv eingestellt werden kann. Jedoch führen diese weicheren und oft gummiartigen Zuckerrüben bei der Verarbeitung (Schneidarbeit und Abpressung) häufig zu deutlichen Problemen (Frenzel 2021). Die in Manuskript IV dargestellten Untersuchungen sollten die Auswirkung von Bewässerung und Trockenstress auf das Ausmaß der Beschädigung prüfen. Da jedoch kein deutlicher Effekt der Bewässerung auf die Festigkeit der Rübe gefunden wurde, konnte auch keine Auswirkung auf die Beschädigungsempfindlichkeit nachgewiesen werden. Somit konnte die Brüchigkeit von Rüben mit hohem Wassergehalt, die in der Praxis beschrieben wird, nicht nachgeprüft werden.

5.3 Festigkeit als Sorteneigenschaft von Zuckerrüben

Während bei vielen Obst- und Gemüsearten die Festigkeit als Sorteneigenschaft züchterisch bearbeitet wird (Abbott 2004), wird diese bei Zuckerrüben bislang nicht berücksichtigt. Für eine effiziente Züchtung ist ein hoher genetischer Einfluss auf den Phänotypen wichtig. Für Ertrags- und Qualitätsparameter von Zuckerrüben konnte der höchste genotypische Einfluss auf die Gehalte an Betain und Zucker ermittelt werden (Hoffmann et al. 2009). Im Gegensatz zu anderen Kulturen wie Getreide zeigte sich bei Zuckerrüben eine hohe Umweltstabilität der Ertrags- und Qualitätsparameter.

Der genotypische Einfluss auf die Festigkeit wurde anhand von Sorten ermittelt, die sich in ihrer Ertragsbildung und in ihrem Verhalten in früheren Lagerungsversuchen unterschieden, ergänzt durch eine alte Sorte. Es zeigte sich, dass der genotypische Einfluss auf die Festigkeit deutlich höher als der bislang bekannte genotypische Einfluss auf Ertrags- und Qualitätsparameter war. Trotz der sehr unterschiedlichen Umwelten war die Interaktion zwischen Genotyp und Umwelt gering und im Verhältnis zum genotypischen Einfluss vernachlässigbar. Nause et al. (2021) konnten diesen stabilen genotypischen Einfluss auch an jungen Zuckerrüben im Gewächshaus feststellen. Die Unterschiede in der Festigkeit von Zuckerrübengenotypen entwickelten sich bereits früh, sodass eine schnellere und effiziente Selektion in Gewächshausversuchen möglich ist. Der Nachweis, dass die genotypischen Unterschiede auch nach der Lagerung von Zuckerrüben zu finden waren, kann für die Verarbeitung von gelagerten Zuckerrüben von Interesse sein. Sorten, deren Festigkeit nach der Ernte für die Verarbeitung vorteilhaft ist, haben diese Eigenschaften auch noch nach der Lagerung. Dieser Zusammenhang erleichtert die Züchtung von Sorten mit vorteilhaften mechanischen Verarbeitungseigenschaften.

Das züchterische Potential der Festigkeit zeigte sich darin, dass bei aktuell marktverfügbaren Sorten erhebliche Unterschiede in der Festigkeit auftraten, obwohl die Festigkeit als Merkmal bisher nicht züchterisch bearbeitet wurde und somit ein zufälliges Produkt ist. Da Sorten mit einem höheren Zuckergehalt tendenziell eine höhere Festigkeit aufwiesen, deutet dies auf eine kausale, wenn vielleicht auch indirekte Beziehung zwischen Festigkeit und Zuckergehalt hin.

5.4 Ursachen für Sortenunterschiede in der Festigkeit von Zuckerrüben

Der Zusammenhang zwischen Zellgröße, Zellwandgehalt und Festigkeit wurde für Kartoffeln und Möhren bereits nachgewiesen (Hudson 1975; Zdunek und Umeda 2005). Für Zuckerrüben konnte nun bestätigt werden, dass Sortenunterschiede in der Festigkeit auf den Zellwandgehalt zurückzuführen sind (Manuskript III), wobei festere Sorten einen höheren Zellwandgehalt und tendenziell auch einen höheren Zuckergehalt aufwiesen. Anhand der Ergebnisse von Milford (1976) lässt sich daher vermuten, dass festere Sorten auch kleinere Zellen haben. Nause et al. (2020) zeigten, dass sich unterschiedliche *Beta* Arten nicht nur in ihrer Zellgröße unterschieden, sondern auch in ihrer Zellgrößenverteilung. Da die charakteristische Struktur der Zuckerrübe bereits in frühen Entwicklungsstadien entsteht, könnten mögliche Unterschiede in der Zellgrößenverteilung zwischen Sorten bereits früh auftreten (Artschwager 1926). Diese Vermutung wurde durch die Ergebnisse von Nause et al. (2021) unterstützt, die herausfanden, dass Unterschiede in der Festigkeit von Zuckerrüben bereits in sehr frühen Wachstumsphasen der Zuckerrübe zu finden sind. Weitergehende Untersuchungen, die den strukturellen Aufbau und die Zellgröße in Verbindung mit der Festigkeit von Zuckerrüben untersuchen, könnten Erklärungsansätze für das abweichende Verhalten der Sorte 2 in dieser Arbeit liefern. Während sich der strukturelle Aufbau und die Anzahl an Kambiumringen trotz des großen Züchtungsfortschritts im Ertrag kaum verändert haben, hat sich der Zellwandgehalt zu Gunsten des Zuckergehalts vermindert (Loel et al. 2014). Diese Verringerung des Zellwandgehalts hat zu einer geringen Festigkeit von aktuellen Zuckerrübensorten im Vergleich zu alten Zuckerrübensorten geführt, mit möglichen Auswirkungen auf die Ernte und Verarbeitung.

Obwohl eine genaue Analyse der Zellwandzusammensetzung von *Beta*-Arten auf Polymer- und Phenolebene keine Erklärungsansätze für die Unterschiede in der Festigkeit liefern konnte (Schäfer et al. 2020), erwiesen sich in dieser Arbeit Sorten mit höherem Pektingehalt in der Zellwand fester als Sorten mit niedrigem Pektingehalt. Die restlichen Bestandteile der Zellwand unterschieden sich zwischen den Sorten und auch zwischen den Umwelten überraschend gering (Manuskript III & IV). Der Pektingehalt ist maßgeblich in der Mittellamelle zwischen einzelnen Zellen zu finden und ist hauptsächlich verantwortlich für die Zelladhäsion in Zuckerrüben (Marry et al. 2006). Die vermutlich höhere Zelladhäsion der Sorten mit höherem Pektingehalt deutet darauf hin, dass bei Krafteinwirkung zuerst die Verbindung zwischen den Zellen bricht und nicht die Zelle selbst. Dahingegen werden Sortenunterschiede im Turgor hauptsächlich bei hohem Turgor relevant, da dann Zellen eher platzen, als dass der Zellverband sich wegen mangelnder Zelladhäsion löst (Harker und Hallett 1992). Der Turgoreffekt scheint in diesen Untersuchungen gering gewesen zu sein; allerdings wurden die Bruchstellen auch nicht intensiv auf die Art des Bruches untersucht. Weitere Untersuchungen der Bruchstellen, insbesondere bei der Kompressionsmessung, können Aufschluss über die Art des Bruchs geben. Sinnvoll erscheint es auch, die Art des Bruches durch mechanische Einwirkung beim Roden und Abreinigen zu vergleichen, um festzustellen, ob diese auf dem gleichen Mechanismus beruhen.

5.5 Einfluss der Festigkeit auf die Beschädigung und Lagerungsverluste

Da Sortenunterschiede deutlicher bei einem hohen Niveau an Lagerungsverlusten auftreten (Kenter und Hoffmann 2009b), war es wegen der insgesamt geringen Lagerungsverluste bislang in Sortenversuchen schwierig, Unterschiede zu erfassen (Kenter und Ladewig 2020). Versuche in Umwelten mit ungünstigen Wachstumsbedingungen und/oder Pathogeninfektionen während der Wachstumsperiode werden in den amtlichen Sortenversuchen wegen der Inhomogenität des Bestandes meistens schon vor der Ernte als ungenügend eingestuft und nicht für die Sortenprüfung berücksichtigt. Daher hatten die Rüben der bei Kenter und Ladewig (2020) in die Auswertung einbezogenen Umwelten nur geringe Lagerungsverluste. Zudem war die Variation der Sorten möglicherweise nur eingeschränkt, da die Züchtungsunternehmen das Prüfsortiment nach ähnlichen Auswahlkriterien selektiert haben. Die Erfassung der Lagerfähigkeit in Sortenversuchen ist dadurch nicht nur schwierig, sondern wegen der zusätzlichen Lagerung auch sehr aufwendig. Es wird daher ein Parameter benötigt, mit dem indirekt und einfach die Lagerfähigkeit von Sorten beschrieben werden kann.

In dieser Arbeit konnte gezeigt werden, dass Sorten mit einer höheren Festigkeit der Rübe in der Tendenz eine geringere Beschädigungsempfindlichkeit und damit geringeren Wurzelbruch aufwiesen. Zudem zeigten sie in Umwelten mit hohen Lagerungsverlusten auch einen geringen Pathogenbefall mit geringeren Lagerungsverlusten. Da der Gehalt an Zellwandbestandteilen die Festigkeit der Rübe maßgeblich bestimmt, scheint es sich bei der Resistenz gegenüber Lagerungspathogenen um eine unspezifische Resistenz durch eine mechanische Barriere des festeren Gewebes zu handeln. Dies wurde bereits von Liebe und Varrelmann (2016) vermutet, da sich zeigte, dass diverse Spezies für den Pathogenbefall verantwortlich waren. Sie folgerten daraus, dass die genotypischen Unterschiede auf einer unspezifischen Resistenz, begründet durch eine besonders widerstandsfähige Zellstruktur, beruhen. Dass sich kleine Parenchymzellen und damit der Zellwandgehalt positiv auf die Lagerfähigkeit von Zuckerrübensorten auswirken, bestätigten auch Madritsch et al. (2020) in aktuellen Untersuchungen.

Die umweltspezifische Festigkeit hatte keine Auswirkung auf die Lagerfähigkeit der Zuckerrüben, infolgedessen können anhand der Festigkeit die erwartbaren Lagerungsverluste für Rüben aus unterschiedlichen Umwelten nicht abgeschätzt werden. Es ist bislang nicht bekannt, warum äußerlich gesunde, ertragreiche und feste Rüben während der Lagerung einen starken Pathogenbefall und hohe Lagerungsverluste aufweisen können. Möglicherweise haben diese Zuckerrüben über den Wurzelbruch und sichtbare oberflächliche Verletzungen hinausgehende tiefere Verletzungen. Auch Quetschungen sind erst nach einer gewissen Zeit zu erkennen. So haben Peterson und Hall (1983) die Beschädigung erst drei Wochen nach der mechanischen Belastung bestimmt, damit Symptome auftreten konnten. Da in dieser Studie die Beschädigung möglichst zeitnah nach der Ernte und damit nach der mechanischen Belastung bestimmt wurde, kann es sein, dass für einige Umwelten die Beschädigung unterschätzt wurde. Sorten reagieren zwar unterschiedlich auf mechanische Belastung, jedoch wird das Ausmaß der Beschädigung maßgeblich von der Rodereinstellung bestimmt (Hoffmann 2018). Es ist zudem möglich, dass Zuckerrüben dieser Umwelten bereits zum Erntezeitpunkt nicht sichtbare Infektionen haben (Campbell und Klotz 2007). Somit eignet sich die Festigkeitsprüfung nicht, um in Zuckerfabriken die Verarbeitungsreihenfolge zu planen.

5.6 Ausblick

Zuckerrübensorten mit geringen Lagerungsverlusten werden zur Verlängerung der Verarbeitungskampagne und damit zur Effizienzsteigerung in der Wertschöpfungskette benötigt. Eine gute Lagerfähigkeit reduziert dabei nicht nur den Zuckerverlust, sondern sichert auch die Qualität für die Verarbeitung. Die Festigkeit als Qualitätsparameter von Zuckerrüben wurde bislang züchterisch nicht aktiv bearbeitet.

Die Ergebnisse dieser Arbeit zeigen, dass sich Zuckerrübensorten in ihrer Festigkeit signifikant unterscheiden und der Sorteneffekt im Vergleich zum Umwelteffekt hoch ist.

Für die Landwirte gäbe die Einführung der Festigkeit als Sorteneigenschaft die Möglichkeit, die Zuckerrübensorten auch nach ihrer Beschädigungsempfindlichkeit und tendenziellen Lagerfähigkeit auszuwählen. Da heute bereits zum Zeitpunkt der Aussaat die Fläche für späte Rodetermine und anschließende Mietenlagerung festgelegt wird, könnte mit der Sortenwahl die Effizienz der gesamten Wertschöpfungskette gesteigert werden. Somit lässt sich vermuten, dass auf Grund der dargestellten Beziehung zwischen Zuckergehalt und Zellgröße sowie dem daraus resultierenden Einfluss auf die Festigkeit, festere Sorten zuckergehaltsbetonte Sorten sind. Zuckergehaltsbetonte Sorten haben meist einen geringeren Zuckerertrag als Sorten mit hohem Rübenertrag (Wolf 1995). Landwirte müssen möglicherweise abwägen, ob der maximale Ertrag oder die Lagerfähigkeit im Vordergrund steht und individuell für jeden Rodetermin entscheiden.

Somit stellt sich für die Züchtung die Frage, ob es möglich ist, diese Beziehung zwischen Zuckergehalt und Festigkeit zu überwinden, oder ob es ausgewogene Sorten mit hohem Ertrag und einer erhöhten Festigkeit gibt. Um diese Frage zu beantworten, muss für die Züchtung ein Zielwert der Festigkeit festgelegt werden. Dieser Zielwert hängt stark von der Beziehung der Festigkeit zur Beschädigungsempfindlichkeit und zu Lagerungsverlusten ab. Möglicherweise gibt es hier eine optimale Festigkeit, über die hinaus es keine weitere positive Auswirkung für die Ernte und Lagerung gibt. Um dieses Optimum zu bestimmen, werden weitere Untersuchungen benötigt, in denen Sorten unterschiedlicher Festigkeiten definiert beschädigt werden. Die züchterische Bearbeitung der Festigkeit wird durch die geringe Interaktion zwischen Genotyp und Umwelt und die Möglichkeit der Selektion in frühen Entwicklungsstadien deutlich einfacher und effizienter.

Auf der anderen Seite kann sich die Festigkeit der Rübe auch auf die Verarbeitung in den Zuckerfabriken auswirken. Auch wenn immer eine Mischung von Rüben aus unterschiedlichen Umwelten und Sorten verarbeitet wird, hat sich die Verringerung der Festigkeit der Zuckerrübensorten mit der Züchtung auf die Verarbeitung in der Fabrik ausgewirkt. So zeigte eine aus den Ergebnissen dieser Arbeit entwickelte Untersuchung der ESST (European Society for Sugar Technology), dass sich die Festigkeit der Sorten signifikant auf die Extraktion und Abpressbarkeit der ausgelaugten Zuckerrübenschnitzel auswirkt (unveröffentlicht). Daher scheint es ebenfalls sinnvoll, eine optimale Festigkeit für die Verarbeitung der Zuckerrüben zu bestimmen.

Bereits in früheren Untersuchungen konnten indirekte Selektionsparamater für die Lagerungseigenschaften, wie den Zellwandgehalt (Markgehalt; (Schnepel und Hoffmann 2016)), ermittelt werden. Die hier gefunden Zusammenhänge zwischen Zellwandgehalt und Festigkeit deuten darauf hin, dass die Selektionsparameter sehr ähnlich sind. Die Bestimmung des Markgehalts kann mit Hilfe von NIRS (near-infrared spectroscopy) relativ genau (Huijbregts et al. 1996) und automatisiert (Meldau et al. 2019) durchgeführt werden. Dies kann für die Züchtung die Möglichkeit bieten, ihren Genpool kostengünstig zu analysieren und in Extreme aufzuteilen. Da der Markgehalt auch nur ein indirektes Merkmal für die Festigkeit ist, scheint es für diese Extreme sinnvoll zu sein, die Festigkeit mit den beschriebenen Methoden zu bestimmen. Hinzu kommt, dass der Markgehalt die Festigkeit auf Ebene der Umwelt nicht ausreichend beschreibt, und somit in den offiziellen Sortenversuchen die Festigkeit zum Vergleich vermutlich besser geeignet ist. Dies wird derzeit in den offiziellen Sortenversuchen geprüft.

Für eine Etablierung der Festigkeit als neues Merkmal in der Sortenprüfung ist es wichtig, weitere Einflussfaktoren auf die Festigkeit zu untersuchen. Der Einfluss von Krankheiten wie Wurzelfäulen, viröser Vergilbung (Hossain et al. 2021) oder SBR (Syndrome Basses Richesses; (Pfitzer et al. 2020)) auf die Festigkeit und dadurch auf die Beschädigungsempfindlichkeit und auf die Verarbeitung der Zuckerrübe ist bislang nicht bekannt.

Weiterhin sollte die Wirkung der Festigkeit auf die Infektion mit Pathogenen genauer untersucht werden. Es konnte bislang nicht geklärt werden, ob eine hohe Festigkeit nur die Infektionsbedingungen für Pathogene verschlechtert, oder ob auch ihre Ausbreitungsgeschwindigkeit innerhalb der Zuckerrübe reduziert ist. Daher sollten an kontrolliert beschädigten Rüben Inokulationsversuche durchgeführt und die Pathogenausbreitung untersucht werden.

Die Einführung der Festigkeit als Sortenmerkmal der amtlichen Sortenprüfung könnte in Zukunft dazu beitragen, die Verluste bei der Ernte, der Lagerung und möglicherweise der Verarbeitung zu reduzieren, wenn Zielwerte definiert werden können und es tiefere Erkenntnisse über die Wirkung der Festigkeit auf die Lagerungsverluste gibt.

6 Zusammenfassung

Eine Verlängerung der Verarbeitungskampagne kann zu einer Effizienzsteigerung in der Zuckerproduktion führen, da die Fixkosten der Zuckerfabriken gesenkt werden. Dies erfordert jedoch eine verlängerte Lagerungsdauer der Zuckerrüben zwischen Ernte und Verarbeitung, was unvermeidbar mit Lagerungsverlusten verbunden ist.

Da diese Lagerungsverluste maßgeblich von Beschädigungen während der Ernte beeinflusst werden, sind neben angepassten Rodereinstellungen auch Sorten mit verringerter Beschädigungsempfindlichkeit erforderlich. Es wird erwartet, dass sich Sorten mit einer geringeren Beschädigungsempfindlichkeit von Sorten mit einer hohen Beschädigungsempfindlichkeit in der Festigkeit unterscheiden. Daher war das Ziel dieser Arbeit, die Festigkeit von Zuckerrüben zu untersuchen und Ursachen für mögliche Sortenunterschiede zu bestimmen. Zudem sollte der Zusammenhang zwischen der Festigkeit, der Beschädigungsempfindlichkeit und den Lagerungsverlusten erforscht werden.

Für Zuckerrüben gab es bisher keine einheitlich detaillierte Methodenbeschreibung zur Erfassung der Festigkeit. Deshalb wurden im Manuskript I die Auswirkungen des Waschens, des Erdanhanges, der Lagerungsdauer vor der Messung, der Messorte und der Stichprobengröße auf Penetrations- und Kompressionstests untersucht. Zusätzlich wurden Biegeversuche durchgeführt, um Empfehlungen zur Versuchsdurchführung abzuleiten. Dazu wurden im Jahr 2017 zwei Zuckerrübensorten im Gewächshaus und vier *Beta*-Genotypen in Feldversuchen angebaut. Mit dem Penetrationstest konnten zwei Festigkeitsparameter bestimmt werden. Die Kraft, die erforderlich ist, um das Periderm der Zuckerrübe zu durchdringen, wurde als Penetrationswiderstand definiert und die durchschnittliche Festigkeit des darunter liegenden Gewebes bis zu einer Tiefe von fünf Millimeter als Gewebefestigkeit. Durch den Kompressionstest wurde die Druckfestigkeit bestimmt, welche die Kraft beim Bruch des Probenzylinders darstellt. Aufgrund des geringen Erdanhanges hatte das Waschen keinen Einfluss auf den Penetrationstest, doch insbesondere bei höherem Erdanhang sollten die Rüben vor der Messung per Hand gewaschen werden. Nach der Wäsche sollte die Messung innerhalb von 48 Stunden durchgeführt werden, da danach ein signifikanter Einfluss auf das Messergebnis auftritt. Die Messposition beeinflusste das Ergebnis des Penetrations- sowie Kompressionstests ebenfalls und muss somit vor der Messung definiert werden. Während die Messergebnisse bei Penetrations- und Kompressionstests auch bei einer geringen Stichprobengröße stabil waren und Sortenunterschiede zwischen den *Beta*-Genotypen erfasst wurden, zeigte der Biegeversuch aufgrund der intensiven Probenvorbereitung eine hohe Messvariabilität.

Für eine effiziente züchterische Veränderung der Festigkeit von Zuckerrüben sind Kenntnisse über die genotypische Variation und den Einfluss der Umwelt auf die Festigkeit relevant. Daher wurde in Manuskript II die Bedeutung von Sorte, Umwelt und deren Interaktion auf die Festigkeit von Zuckerrüben bestimmt. Außerdem wurde die Bandbreite der Festigkeit der in Deutschland verfügbaren Zuckerrübensorten untersucht. Hierfür wurden in den Jahren 2018 und 2019 sechs unterschiedliche

Zuckerrübensorten in sieben Umwelten in Deutschland angebaut und im Jahr 2020 ein Genotypenscreening mit zwölf marktverfügbaren Sorten in einer Umwelt in Deutschland durchgeführt. Der Effekt der Sorte auf die Festigkeit war deutlich höher als auf die Ertrags- und Qualitätsparameter. Da die Interaktion zwischen Genotyp und Umwelt unbedeutend war, ist eine züchterische Selektion auf Basis von wenigen Umwelten möglich. Die marktverfügbaren Sorten zeigten ebenfalls große Unterschiede in der Festigkeit, wobei Sorten mit einem höheren Zuckergehalt in der Tendenz eine höhere Festigkeit aufwiesen. Diese Unterschiede lassen vermuten, dass sich die Festigkeit auf die Beschädigungsempfindlichkeit und damit auf die Lagerungsverluste auswirkt und in der Konsequenz die Verarbeitung in der Zuckerfabrik beeinflusst.

Daher wurde in Manuskript III der Einfluss der Festigkeit auf die Beschädigungsempfindlichkeit und Lagerungseigenschaften überprüft und ihre Veränderungen während der Lagerung erfasst. Zudem sollten Ursachen für genotypische Unterschiede in der Festigkeit untersucht werden. Dazu wurden in den Jahren 2018 und 2019 Feldversuche mit sechs Zuckerrübensorten in sieben Umwelten in wichtigen Regionen des deutschen Zuckerrübenanbaus angelegt und im Anschluss am Institut für Zuckerrübenforschung in Göttingen in Klimacontainern unter kontrollierten Bedingungen gelagert. Zusätzlich wurden zwölf marktverfügbare Sorten in einer Umwelt im Jahr 2020 angebaut. Eine Ursache für die genotypischen Unterschiede in der Festigkeit war der alkoholunlösliche Zellwandgehalt (AIR). Dieser bestimmte mit einem Bestimmtheitsmaß von bis zu 0,97 die Druckfestigkeit, während sich die Zusammensetzung der Zellwand zwischen den Sorten kaum unterschied. Während der Lagerung stiegen der Penetrationswiderstand und die Druckfestigkeit unabhängig von der Sorte an. Daher können die Auswirkungen auf die Prozesse nach der Lagerung im Voraus durch eine Analyse der frischen Zuckerrüben abgeschätzt werden.

Sorten mit einer höheren Festigkeit wiesen tendenziell einen geringeren Wurzelbruch nach der Ernte und daraus folgend geringe Lagerungsverluste auf. Jedoch traten genotypische Unterschiede in den Lagerungsverlusten nur in Umwelten mit hohen Lagerungsverlusten auf.

Unter den Faktoren, die während des Anbaus beeinflusst werden können, haben die Stickstoffdüngung und die Wasserverfügbarkeit den größten Einfluss auf die Zusammensetzung der Zuckerrüben. Der Einfluss dieser Faktoren auf die Festigkeit und die Zusammensetzung der Zuckerrübensorten sowie die Auswirkungen auf Wurzelbruch und Lagerungsverluste sollten im Manuskript IV untersucht werden. Zu diesem Zweck wurden 2018 und 2019 Feldversuche mit drei Zuckerrübensorten und drei Stickstoffversorgungsstufen in den Niederlanden und Belgien sowie mit drei Bewässerungsvarianten in Schweden durchgeführt. Der Einfluss von Stickstoff- und Wasserversorgung auf die Festigkeit war deutlich geringer als der Einfluss der Sorten. Auch auf den Zellwandgehalt hatten die Umwelten und die Sorten den größten Einfluss, während die Zellwandzusammensetzung nur geringe Unterschiede aufwies. Während eine steigende Stickstoffversorgung keine Auswirkung auf den Wurzelbruch und Zuckerverlust nach der Lagerung hatte, war der Invertzuckergehalt nach der Lagerung bei hoher Stickstoffdüngung etwas geringer. Die Wasserverfügbarkeit hatte keinen Effekt auf den Wurzelbruch. Jedoch hatten Rüben aus Parzellen, die über das Jahr mehrmals beregnet wurden, einen deutlich höheren Lagerungsverlust. Dies ist möglicherweise durch einen erhöhten Krankheitsdruck verursacht worden.

Im Rahmen dieser Arbeit war es möglich, eine detaillierte Methode zur Bestimmung der Festigkeit von Zuckerrüben zu entwickeln und zu zeigen, dass sich Zuckerrübensorten signifikant in der Festigkeit unterscheiden. Diese Sortenunterschiede treten dabei unabhängig von der Umwelt auf und erlauben eine Bestimmung der Festigkeit von Sorten anhand von wenigen Umwelten. Für Zuckerrübensorten scheint zu gelten, dass ein höherer Zellwandgehalt zu einer höheren Festigkeit führt und damit zu geringerer Beschädigung bei der Ernte, was wiederum zu geringeren Lagerungsverlusten führen kann. Eine Einführung der Festigkeit als Sortenmerkmal für die Züchtung und in die amtliche Sortenprüfung könnte somit dazu beitragen, Ernte- und Lagerungsverluste von Zuckerrüben in Zukunft zu verringern und damit auch zu einer Effizienzsteigerung der Zuckerproduktion beizutragen.

7 Summary

Extending the processing campaign can increase efficiency in sugar production by reducing sugar factories' fixed costs. However, this requires an extended storage period for the sugar beet between harvest and processing, which inevitably involves storage losses.

Since these storage losses are significantly influenced by damage during harvest, varieties with reduced damage susceptibility are required in addition to adapted harvester settings. It is expected that varieties with low damage susceptibility will differ from varieties with high damage susceptibility in their tissue strength. Therefore, the objective of this work was to investigate sugar beet tissue strength and determine causes for possible varietal differences. In addition, the relationship between tissue strength, damage susceptibility and storage losses should be investigated.

For sugar beet, there has been no uniform detailed method description for measuring texture properties. Therefore, in Manuscript I, the effects of washing, soil tare, storage time before measurement, measurement position, and sample size on puncture and compression tests were investigated. In addition, bending tests were conducted to derive recommendations on test procedure. For this purpose, two sugar beet varieties were grown in the greenhouse and four *Beta* varieties were grown in field trials in 2017. The puncture test determined two texture parameters. The force required to penetrate the sugar beet periderm was defined as puncture resistance, and the average strength of the underlying tissue to a depth of five millimeters was defined as tissue firmness. The compression test was used to determine the compressive strength, which is the force at rupture of the sample cylinder. Due to low soil tare, washing had no effect on the puncture test, but especially in the case of higher soil tare, the roots should be washed by hand before measurement. After washing, the measurement should be performed within 48 hours, because after that time a significant influence on the measurement result occurred. The measurement position also influenced the result of the puncture as well as compression test and thus has to be defined before the measurement. While the measurement results of the puncture and compression test were stable with a small sample size and variety differences between the *Beta* varieties were determined, the bending test showed a high measurement variability due to the intensive sample preparation.

For efficient breeding modification of sugar beet tissue strength, knowledge of genotypic variation and the influence of environment on tissue strength is relevant. Therefore, the importance of genotype, environment and their interaction on sugar beet tissue strength was determined in Manuscript II. Furthermore, the range of tissue strength of sugar beet varieties available in Germany was investigated. For this purpose, six different sugar beet genotypes were grown in seven environments in Germany in 2018 and 2019, and a genotype screening with 12 commercial genotypes was conducted at one location in Germany in 2020. The effect of genotype on tissue strength was significantly higher than on yield and quality parameters. Since the interaction between genotype and environment was negligible, breeding selection based on a few locations is possible. Commercial genotypes also showed large differences in tissue strength, with genotypes with higher sugar content tending to have

higher tissue strength. These differences in tissue strength suggest that tissue strength affects damage susceptibility and thus storage losses and, as a consequence, influences processing in the sugar factory.

Therefore, in Manuscript III, the influence of tissue strength on damage susceptibility and storage characteristics was examined and its changes during storage were recorded. In addition, reasons for genotypic differences in tissue strength should be investigated. Therefore, field trials with six sugar beet genotypes were established in seven environments in important regions of German sugar beet cultivation in 2018 and 2019 and subsequently stored in climate containers under controlled conditions at the Institute of Sugar Beet Research in Göttingen. In addition, 12 commercial genotypes were grown at one location in 2020. One reason for the genotypic differences in tissue strength was the alcohol-insoluble cell wall content (AIR). This determined compressive strength with a coefficient of determination of up to 0.97, while cell wall composition differed little between genotypes. During storage, puncture resistance and compressive strength increased regardless of genotype. This characteristic allows to estimate the effects on the post-storage processing by analyzing the fresh sugar beets in advance.

Genotypes with higher tissue strength tended to have lower postharvest root tip breakage and resulting in lower storage losses. However, genotypic differences in storage losses occurred only in environments with high storage losses.

Among the factors that can be influenced during cultivation, nitrogen application and water availability have the greatest influence on sugar beet composition. The influence of these factors on the tissue strength and composition of sugar beet varieties, as well as the effects on root tip breakage and storage losses, should be investigated in Manuscript IV. For this purpose, field trials were conducted in 2018 and 2019 with three sugar beet varieties and three nitrogen doses in the Netherlands and Belgium and with three irrigation treatments in Sweden. The influence of nitrogen and water supply on tissue strength was significantly lower than the influence of varieties. Environments and varieties also had the greatest influence on cell wall content, while cell wall composition showed only minor differences. While increasing nitrogen supply had no effect on root tip breakage and sugar loss after storage, invert sugar content after storage was slightly lower under high nitrogen fertilization. Water availability had no effect on root tip breakage, but plots irrigated several times throughout the year had significantly higher storage loss, possibly due to increased disease pressure.

In this work, it was possible to develop a detailed method for determining the tissue strength of sugar beets and to show that sugar beet varieties differ significantly in tissue strength. These varietal differences occur independently of the environment and allow determination of the tissue strength of varieties based on a small number of locations. For sugar beet varieties, it appears that higher cell wall content leads to higher tissue strength and thus less damage at harvest, which in turn may lead to lower storage losses. Introducing tissue strength as a variety characteristic for breeding and official

variety testing could thus help to reduce storage losses of sugar beet in the future and also contribute to an increase in the efficiency of sugar production.

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Veröffentlichungen und Vorträge während der Promotion

Veröffentlichte Manuskripte

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